EVALUATION OF SERUM HOMOCYSTEINE AS PROGNOSTIC MARKER OF ORAL SUBMUCOUS FIBROSIS

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CERTIFICATE BY THE GUIDE

This is to certify that **Dr.M.GAYATHRI**, Post graduate student (2014-2017) in the Department of Oral Medicine and Radiology (Branch IX) , Tamil Nadu Government Dental College and Hospital, Chennai 600003, has done this dissertation titled "**EVALUATION OF SERUM HOMOCYSTEINE AS PROGNOSTIC MARKER OF ORAL SUBMUCOUS FIBROSIS"** under my direct guidance and supervision in partial fulfillment of the M.D.S. degree examination in April 2017 as per the regulations laid down by Tamilnadu Dr. M.G.R. Medical University, Chennai 600032 for **M.D.S., Oral Medicine and Radiology (Branch – IX)** degree examination.

GUIDE:

Prof. Dr. G.V. MURALI GOPIKA MANOHARAN, M.D.S,

Professor, Department of Oral Medicine and Radiology,

Tamil Nadu Government Dental College and Hospital,

Chennai 600003.

.

CERTIFICATE BY HEAD OF THE DEPARTMENT / HEAD OF THE INSTITUTION

This is to certify that the Dissertation entitled "**EVALUATION OF SERUM HOMOCYSTEINE AS PROGNOSTIC MARKER OF ORAL SUBMUCOUS FIBROSIS"** is a bonafide work done by **Dr.M.GAYATHRI,** Post Graduate student (2014-2017) in the Department of Oral Medicine and Radiology under the guidance of **Prof. Dr. G.V. MURALI GOPIKA MANOHARAN, MDS.,** Professor, Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003.

Dr. S. JAYACHANDRAN MDS., Ph.D., MAMS., MBA.,
Professor and Head of the Department,
Department of Oral Medicine and Radiology,
Tamil Nadu Government Dental College & Hospital,
Chennai - 600 003.

Dr. B. SARAVANAN MDS., Ph.D.,
Principal,
Tamil Nadu Government Dental College & Hospital,
Chennai - 600 003.

DECLARATION BY THE CANDIDATE

Title of the study	Evaluation Of Serum Homocysteine As Prognostic Marker Of Oral Sub Mucous Fibrosis
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Name of the guide	PROF. DR. G.V. MURALI GOPIKA MANOHARAN MDS.,
Head of the department	PROF. DR. S. JAYACHANDRAN MDS., PH D., MAMS., MBA

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Signature of the Candidate

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ABSTRACT

Background: Oral submucous fibrosis (OSF) is a chronic progressive premalignant condition of the oral cavity associated with significant morbidity. Wide range of investigations have been carried out in this condition to identify the causation and pathogenesis. Biochemical investigations have been the earliest form of intervention to localize the parameters that predispose to the development of the condition and prognosticate on its malignant transformation potential. Only few studies have been done to estimate homocysteine level before treatment in oral submucous fibrosis. Hence the present study was conducted to evaluate the serum homocysteine level in oral submucous fibrosis in various stages before and after medical intervention and to compare and assess the serum homocysteine level as a prognostic marker in oral submucous fibrosis.

Aim: To evaluate the serum homocysteine level as prognostic marker in oral submucous fibrosis.

Objective: To estimate serum homocysteine in patients with oral submucous fibrosis in various stages before and after treatment. To compare the serum homocysteine level before and after treatment and assess the value of serum homocysteine level as a prognostic marker.

Methods: A total of 37 study participants comprising of 30 OSF patients and 7 healthy controls were included in the study. OSF patients were graded clinically into 4 grades. All the participants were subjected to homocysteine evaluation. OSF patients were treated with intralesional steroid and supplemental medication for 6 weeks and evaluated for improvement in burning sensation, mouth opening and post treatment Homocysteine. All the values were statistically analysed and the results were drawn.

Abstract

Results: The average age of study participants was 31.59±7.588 years. Majority of OSF patients were males. The mean serum homocysteine level in male and female are 19.82µmol/L and 8.86µmol/L. The mean serum homocysteine level in control group (8.40±1.74µmol/L) and OSF group (20.67±11.26µmol/L) showed statistically significant difference. With progression of disease from Grade I (10.69±2.26µmol/L) to Grade IV (35.17±13.90µmol/L), a statistically significant increase in mean serum homocysteine level was observed. The post treatment mean serum homocysteine levels in OSF patients was 14.76±6.45µmol/L. There was significant reduction in mean serum homocysteine level in OSF patient after treatment was noted (p=0.003). On comparision of mean pre treatment and post treatment serum homocysteine showed statistically significant difference. Statistically significant correlation between reduction in serum homocysteine and improvement in mouth opening was noted.

Conclusion: Hence, Serum Homocysteine level may be contributory as a potential prognostic marker in treatment of Oral submucous fibrosis.

Key words: *Oral submucous fibrosis, Homocysteine, Inflammation, Potentially malignant disorder, Premalignant condition*

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BQ	Betel quid
DNA	Deoxyribonucleic acid
Hcy	Homocysteine
HHcy	Hyperhomocysteinemia
HNSCC	Head and Neck Squamous Cell Carcinoma
IL 1	Interleukin 1
IL-6	Interleukin 6
INF-γ	Interferon Gamma
JNK	c-Jun N-terminal kinases
LOX	Lysyl Oxidase
MTHFR	Methylenetetrahydrofolate reductase
NF-κB	Nuclear factor kappa -light-chain enhancer of activated B cells
OSF	Oral Submucous Fibrosis
OSCC	Oral Squamous Cell Carcinoma
PMD	Potentially Malignant Disorder
ROS	Reactive Oxygen Species
SST	Serum Separating Tube
TNF	Tumor Necrosis Factor
TGF-β	Transforming Growth Factor - Beta
t(Hcy)	Total homocysteine
VAS	Visual Analogue Scale

LIST OF TABLES

LIST OF CHARTS

LIST OF PHOTOGRAPHS

INTRODUCTION

Homocysteine is a sulphur containing aminoacid formed during methionine metabolism during the conversion of methionine to cysteine. Du Vigneud an American biochemist in the early 1930's discovered a structure similar to cysteine with an extra carbon atom and named it as homocysteine. It was studied for many years only in context with the hereditary disease homocystinuria[1].

Clinical researches done over the last decade has shown that elevated levels of homocysteine have been identified as an independent risk factor for atherosclerosis, cardiovascular disease, cerebrovascular disease, renal disease, ischemic optic neuropathy and cancer[2]. Various studies done in oral mucosal diseases such as oral lichen planus[3], burning mouth syndrome[4], atrophic glossitis[5], recurrent aphthous stomatitis[6], Behcets disease[7] and head and neck cancers[8] also reported elevated levels of homocysteine.

The factors contributing to hyperhomocysteinemia include lifestyle changes (vegans, betel nut chewing, smoking, and alcohol), drugs (anti-epileptics) and genetic defects (MTHFR polymorphism).The mechanism of action by which homocysteine causes disease is complex and not completely understood, but mainly explained on the basis of oxidative damage and protein homocysteinylation[9].

Hyperhomocysteinemia exert its detrimental effects through induction of acute and chronic inflammation pathway such as endothelial dysfunction, leukocyte adhesion, oxidative stress and reduction in nitric oxide bioavailability. It has been reported that oxidative stress derived from hyperhomocysteinemia will induce chronic inflammation via the regulation of NF-κB transcription factor. Thus it is noted that

hyperhomocysteinemia is not only produced from inflammation but also the oxidative stress generated from hyperhomocysteinemia will again promote inflammation[10].

Inflammation promotes cell proliferation at the expense of excess amount of vitamins and deficiency of folic acid, vitamin B12 and vitamin B6 is reflected as hyperhomocysteinemia as they are involved in the metabolism of homocysteine. Thus hyperhomocysteinemia indicates the presence of inflammation and indirectly reflects vitamin status[10].

Alteration in homocysteine levels may cause imbalance among the products of the anti-oxidant pathway resulting in increased oxidative metabolism. Homocysteine synthesis is also related indirectly to DNA methylation, a mechanism for gene expression control in normal and tumor cells, thus, showing its contribution in carcinogenesis[11].

Schwartz in 1952 described a condition affecting the oral mucosa including the palate and faucial pillar, called "atrophia idiopathica (tropica) mucosae oris" among five Indian women from Kenya[12]. Later the term “Oral submucous fibrosis” was coined by S.G Joshi in 1953[13]. Paymaster (1956) observed the precancerous nature of OSF, because of the slow onset of squamous cell carcinoma in one third of OSF patients[14].

In 1966 Pindborg and Sirsat, defined OSF as “an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat”[15].

The disease is predominantly seen in southern parts of the subcontinent with a reported prevalence ranging up to 0.4% in Indian rural population[16].There is increased

incidence to 6.42% in the recent years [17]. Majority of them belonged to the age group of 20-40 years of age with male predominance[18]. The condition has been described as a "potentially malignant disorder" in view of the high rate of malignant transformation in the range of 7–13% [19].

The exact etiology of OSF is not well understood. The pathogenesis of the disease is believed to be multifactorial. Numerous factors trigger the disease process by causing a juxtaepithelial inflammatory reaction in the oral mucosa. These include areca nut chewing, ingestion of chillies, genetic and immunologic processes, nutritional deficiencies etc. As it is of multifactorial etiology, no satisfactory treatment has been described in literature till date.

Current evidence suggests that arecoline in the areca nut is the key factor in initiating the disease[20]. Areca nut is the fourth most addictive substance in the world. The habit of betel quid chewing is widespread throughout India and South East Asia[21] and it is widely prevalent in teenagers and young adults[19].

The alkaloids and flavonoids (arecoline, arecaidaine, tannins and catechins) stimulate collagen synthesis and proliferation of fibroblasts and can act both as chemical and physical irritant to oral mucosa. The micro trauma produced by friction of coarse fibres of arecanut facilitates diffusion of alkaloids into the subepithelial connective tissue resulting in juxtaepithelial inflammatory cell infiltration[22].

The ingredients of arecanut stimulates connective tissue growth factor in buccal mucosal fibroblasts through generation of reactive oxygen species (ROS) which strongly activate NF-kappa B, JNK and p38 and damages the cell structures[23].

Moreover vitamin deficiency and malnutrition can derange the repair of the inflamed oral mucosa, leading to defective healing and the resulting atrophic oral mucosa is more susceptible to the effects of areca nut[24].

Wide ranges of investigations have been carried out in this condition to identify the causation and pathogenesis. Biochemical investigations of blood, serum and tissues have been the simple, minimally invasive and earliest form of interventions. Such investigations have largely helped to localize parameters that predispose to the development of the condition, modify its behavior, monitor the response to therapy and prognosticate on its progression and malignant transformation potential[25].

Amino acids and their derivatives can be useful 'molecular/disease markers' as they reflect the protein metabolism, problems related to dietary uptake and aid in understanding the metabolic derangements that occur during the pathological processes induced by the PMDs. They may help to categorize PMDs, their risk for malignant transformation and may aid in its early detection and management[26].

Few studies have shown an association of serum homocysteine in oral mucosal diseases, with reports showing positive correlation and elevated homocysteine levels. The current study is only a fourth of its kind, to evaluate the serum homocysteine levels in OSF and is the first to compare and analyze the serum homocysteine levels before and after medical intervention. Thus this study aimed to assess the homocysteine levels in various grades of progression of OSF, to compare the levels between the different groups before and after medical intervention. Thus this study is intended to evaluate whether homocysteine can be used as a therapeutic prognostic marker in oral submucous fibrosis patients.

AIM AND OBJECTIVE

AIM:

To evaluate the serum homocysteine level as prognostic marker in oral submucous fibrosis.

OBJECTIVE:

1. To estimate serum homocysteine in patients with oral submucous fibrosis in various stages before and after treatment.
2. To compare the serum homocysteine before and after treatment and assess the value of serum homocysteine level as prognostic marker.

REVIEW OF LITERATURE

HISTORY:

OSF has been well established in Indian medical literature since the time of **Susrutha,** a renowned Indian physician, who described a condition resembling OSF under mouth and throat disease as Vidari during the period of 2500-3000 BC. It was characterized by "a progressive narrowing of mouth, depigmentation of oral mucosa and pain on taking food"[15].

Schwartz (1952)[12] for the first time reported a case of "Atrophia Idiopathica (Tropica) Mucosae Oris" occurring in five Indian women living in Kenya and described blanching and stiffness of the oral mucosa, difficulty in opening the mouth and inability to tolerate spicy food.

Joshi (1953)[13] an ENT surgeon described the disease for the first time in India and coined the term "Submucous fibrosis of the palate and pillars".

Different authors have described oral submucous fibrosis in different names as follows: **Lal D (1953)**[27] -"Diffuse oral submucous fibrosis"**, Su I.P (1954)**[28] - "Idiopathic scleroderma of the mouth"**, Behl P.N (1962)**[29]- "Sclerosing stomatitis", **Rao A.B.N (1962)**[30] – Idiopathic palatal fibrosis, **Pindborg J.J (1966)**[15] – Juxta epithelial fibrosis**, Ramanathan K (1981)**[31] – Asian Sideropenic Dysphagia.

The term "**oral submucous fibrosis"** has been widely accepted over the years as it implies the nature of the condition in a simplified form [32].

DEFINITION:

Pindorg J.J, Sirsat S.M (1996)[15] defined oral submucous fibrosis as, "an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always

associated with juxtaepithelial inflammatory reaction followed by fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat."

WHO (1984)[33] had subsequently defined OSF as "a slowly progressive disease in which the fibrous bands form in the oral mucosa, ultimately leading to severe restriction of movement of the mouth, including the tongue."

EPIDEMIOLOGY:

Pindborg JJ et al (1968)[16] in their epidemiological survey concluded that there is an increased incidence of OSF of about 0.18%-1.2% in urban population when compared with 0.04% - 0.4% in rural population.

Ranganathan K et al (2004) [34]conducted a study in Chennai, South India, reported a mean age of 32.4±10.4 years and median age of 29 years. The youngest and oldest ages of occurrence of OSF in this study was 16 and 76 years in males and 24 and 57 years in females. The male to female ratio was 9.9:1.

Ahmad MS et al (2006)[35] carried out an etiological and epidemiological study in 157 cases of OSF in Patna, Bihar, India over a period of 2 years. Maximum number of cases belonged to 21 – 40 years with the youngest recorded case in a 11 year old and oldest one being of 54 years of age.

Hazarey et al (2007)[17] conducted a hospital-based cross-sectional study on various habit patterns associated with OSF in 1,000 cases in Nagpur, Central India, over a 5-year period. the mean age for men was 27.60 ± 9.58 years and for women, 34.78 ±12.21 years and the severity of OSF was more prevalent in women than men even though the male: female ratio was 4.9:1.

Kumar KK et al (2007)[36] studied 75 cases of OSF in Chennai, South India

and found that half of the study population belonged to the age group of 20-29 years.

Angadi PV et al (2011)[37] carried out a review on 205 cases in southern India diagnosed between January 1989 and June 2005 over a period of 16 years. The age range was 14 – 78 years, with mean age being 46 years. OSF was most frequent in the age range of 21- 30 years (47.8%). The overall male to female ratio was around 11:1 with a general trend towards male preponderance.

Nigam NK et al (2014)[38] conducted a survey in east, west, north and south zones of Moradabad district, Uttar Pradesh for a period of one year among 1000 habitual chewers. The prevalence of OSF was found to be 6.3% with a male to female ratio of 6.88:1.

Recent epidemiological data indicates that the number of cases of OSF has raised rapidly in India from an estimated 2, 50,000 cases in 1980 to 2 million cases in 1993. The reasons for rapid increase of the disease are reported due to an upsurge in the popularity of commercially manufactured areca nut preparations (pan masala) in India. There is increased uptake of this habit by young people with male predominance. This could be due to the easy availability of the products in all the places with effective price and marketing strategies[39].

ETIOPATHOGENESIS:

Lal DC (1953)[27] in his study observed that all cases of oral submucous fibrosis gave a history of chewing supari. Supari is the Hindi word of betel nut, which is the fruit of areca catechu palm, which is widely available in India.

Sirsat SM and Khanolkar VR (1962)[22] histologically studied the effect of arecoline on the palate and buccal mucosa of 20 light wistar rats and found arecoline an active ingredient of betel nut played an important role in causation of submucous

fibrosis.

Gupta PC et al (1966)[40] stated that oral use of any tobacco product like gutkha contain arecanut and several other substances in powdered or granulated form which causes oral submucous fibrosis

Wahi PN et al (1966)[41] in their study reported OSF to be higher in patients with poor nutritional status. They found that the patients with OSF showed a higher frequency of deficiency of vitamin A, B, C and multiple vitamins.

Phatak AG (1978)[42] did a study on 34 patients suffering from OSF and observed that these patients had significantly elevated levels of serum globulin and immunoglobulin IgG, stating the possibility of OSF being an auto-immune disorder.

Shiau (1979)[43] done a study in 35 OSF cases amongst Chinese in Taiwan and observations suggested that betel nut chewing was a significant contributory factor and tobacco, hot spicy food and liquor were not important etiologic factors for the occurrence of OSF.

Ramanathan K (1981)[31]observed a prolonged iron and vitamin B complex deficiency in 10 out of 13 submucous fibrosis patients and gave a hypothesis that submucous fibrosis could be the Asian version of Siderophenic Dysphagia.

Caniff JP et al (1981)[44] proved that arecanut can act as potent stimulator for collagen synthesis in human fibroblast culture leading to excessive accumulation of collagen leading to fibrosis.

Canniff JP et al (1986)[45] suggested that the addition of slaked lime to areca nut hydrolyses arecoline to arecaidine and the inflamed mucosa had enhanced permeability to arecoline and arecaidine.

Rajendra R et al (1986)[46] in the study compared the cell mediated and humoral immune response of 50 oral submucous fibrosis patients with 50 leukoplakia, 50 oral cancer and 50 normal patients. The number of high affinity rosette forming cells (HAFC) was found to be significantly decreased and levels of serum IgA, IgD and IgE were found to be elevated both in OSF and oral cancer indicating oral submucous fibrois can be intermediary state in the malignant transformation of normal cells.

Seedat HA et al (1988)[47] studied the clinical and histological features in 14 subjects who had given up betel nut chewing 13 years before but these patients had signs and symptoms suggestive of OSF. It was the concluded that once OSF was induced by the habit of chewing betel nut, the reversal of the disease after cessation of habit did not occur.

Sinor PN et al (1990)[48] demonstrated the dose-response relationship between degree of exposure and the risk of a disease is an important criterion for causal inference. In a case-control study from India, the relative risk increased with the duration as well as the frequency of the areca nut chewing habit.

Jeng JH (1994)[49] conducted a study to observe the pathobiological effects of aqueous extracts of betel quid constituents.lt showed that betel quid contained not only genotoxic and cytotoxic agents but also compounds that had the ability to stimulate cellular proliferation.

Maher R et al (1994)[50] from a case control study in Karachi showed that daily consumption of pan appeared to have important effect than the duration of habit. They suggested that relatively short exposure is sufficient to induce fibrosis in subjects who are susceptible to the disease.

Haque MF et al (1998)[51]in their immunohistochemical study of oral

submucous fibrosis showed the increased evidence of CD4 and HLA-DR positive cells in oral submucous fibrosis, the presence of these immunocompetent cells and high ratio of CD4 to CD8 in oral submucous tissue suggests an ongoing cellular response leading to a possible role of immunity in OSF.

Chaturvedi VN et al (1998)[52] studied 18 cases of oral submucous fibrosis and concluded that increased level of serum IgG in all grades of oral submucous fibrosis suggesting and autoimmune basis of oral submucous fibrosis.

Trivedy C et al (1999)[53] in their study observed that copper is released from areca products during chewing and is deposited in oral tissues. They found that lysyloxidase activity is upregulated in OSF patients. From these findings they hypothesized that cellular events lead to cross linking of collagen and elastin, making them less degradable. The up regulation of lysyloxidase in OSF may be an important factor in the pathogenesis of this disorder.

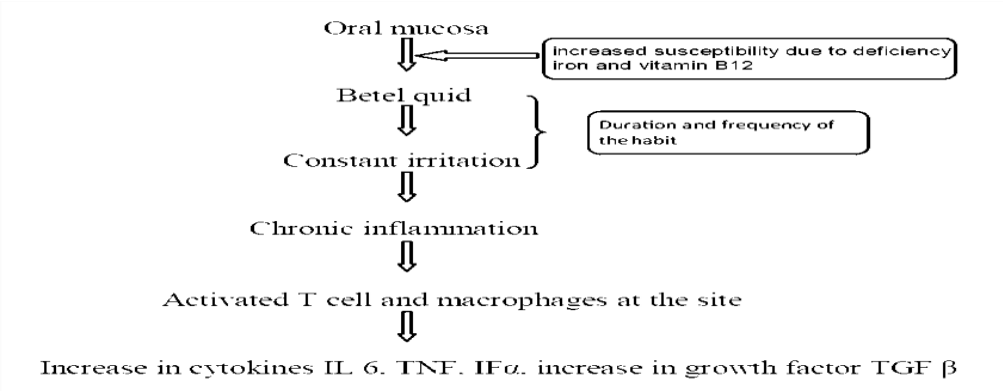
Haque MF et al (2000)[54] demonstrated increased levels of proinflammatory cytokines and reduced antifibrotic interferon γ (IFN-γ) in patients with oral submucous fibrosis which may be the central to the pathogenesis of oral submucous fibrosis.

Chiang et al (2002)[55] showed the betel quid placed in the buccal vestibule for about 15 minutes to an hour and repeated 5 to 6 times a day which leads to constant contact between the mixture and oral mucosa. The alkaloids from the quid are absorbed into the mucosa and undergoes metabolism. Microtrauma produced by the friction of coarse fibers of areca nut also facilitates diffusion of the alkaloids into the subepithelial connective tissue resulting in juxtaepitheial inflammatory cell infiltration.

Nair et al (2004)[56] showed that continuous local irritation by pan masala, gutkha or areca nut can lead to injury related chronic inflammation, oxidative stress and

cytokine production. Oxidative stress and subsequent Reactive oxygen species (ROS) generation can induce cell proliferation, cell senescence or apoptosis, depending upon the level of ROS production. During chronic exposure, these events can lead to preneoplastic lesions in the oral cavity and subsequently to malignancy.

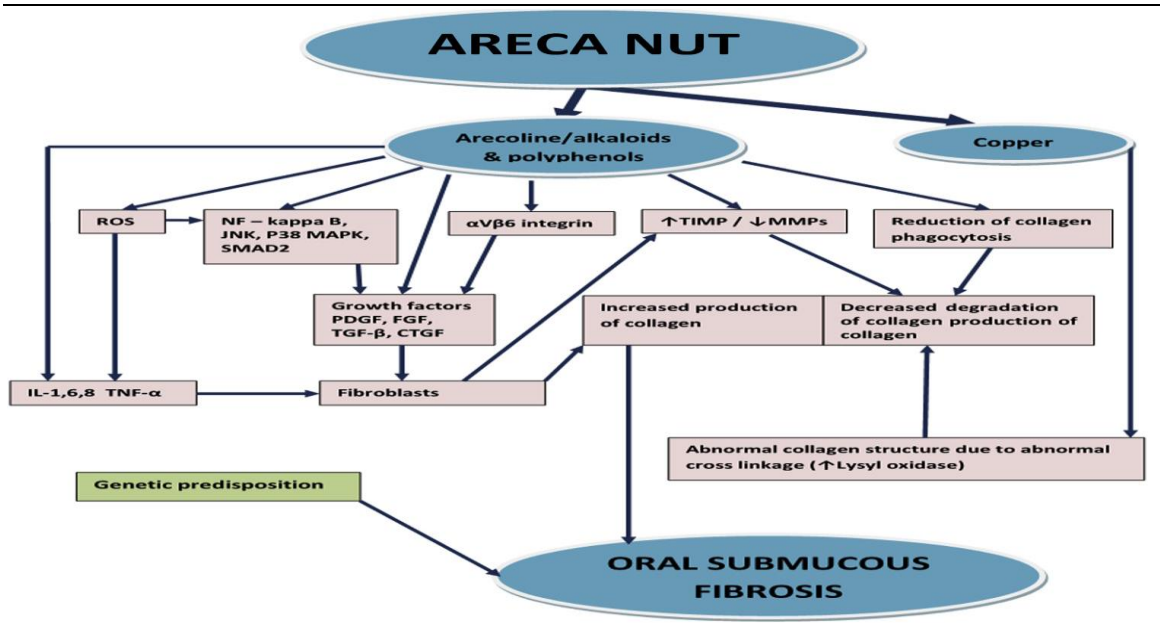
Rajalalitha et al (2005)[57] in the review mentioned the initial events in the disease process in oral mucosa which is in direct contact with the betel quid due to the habit which is the site of constant irritation



Goel et al (2010)[58] found that the patients who used pan masala with a greater frequency per day developed OSF earlier. Daily consumption was more significant than the total duration of the habit.

Wang YP et al (2015)[59] conducted a study in a 68 male OSF patients to evaluate anemia, hematinic deficiencies, and serum gastric parietal cell antibody (GPCA) positivity and found 7.4%, 20.6%, 50.0%, 41.2%, and 13.2% had Hb, iron, vitamin B12 and folic acid deficiencies, and serum GPCA positivity.

Tilakaratne et al (2016)[23] in the review on the etiology and etiopathogenesis of OSF showed the possible biochemical and molecular events known in the pathogenesis of OSF



CLASSIFICATIONS OF OSF[60]

Several classifications based on clinical and histological features, have been put forth by various researchers, based on different aspects of OSF.

A. Classifications based on clinical features of OSF given by: JV Desa (1957),Pindborg JJ (1989), SK Katharia et al (1992), Lai DR et al (1995), R Maher et al (1996), Ranganathan K et al (2001), Rajendran R (2003), Nagesh and Bailoor (2005), Tinky Bose and Anita Balan (2007), Kiran Kumar et al (2007), Chandramani More et al (2011)

B. Classifications based on histopathological features given by: Pindborg JJ and Sirsat SM (1966), Utsunomiya H et al (2005), Kiran Kumar et al (2007)

C. Classification based on clinical and histopathological features: Khanna JN et al (1995)

1. Classification based on clinical features of OSF:

1. JV Desa (1957) divided OSF into three stages as follows:

Stage I : Stomatitis and vesiculation

Stage II : Fibrosis

Stage III : As its sequelae

2. Pindborg JJ in 1989 divided OSF into three stages as follows:

Stage I: Stomatitis includes erythematous mucosa, vesicles, mucosal ulcers, melanotic mucosal pigmentation and mucosal petechiae.

Stage II: Fibrosis occurs in healing vesicles and ulcers, hallmark of this stage.

- Early lesions show blanching of the oral mucosa.
- Older lesions include vertical and circular palpable fibrous bands in the buccal mucosa and around the mouth opening or lips.
- This results in a mottled marble like appearance of the mucosa because of the vertical thick, fibrous bands in association with a blanched mucosa.
- Specific findings include reduction of mouth opening, stiff and small tongue, blanched and leathery floor of the mouth, fibrotic and depigmented gingiva, rubbery soft palate with decreased mobility, blanched and atrophic tonsils, shrunken bud like uvula and sunken cheeks, not commensurate with age or nutritional status.

Stage III: Sequelae of OSF are as follows:

- Leukoplakia is found in more than 25% of individuals with OSF. - Speech and hearing deficit may occur because of involvement of tongue and the Eustachian tube.

3. SK Katharia et al (1992) have given different scores assigned to the patients on the basis of mouth opening between upper and lower central incisors as follows

Score 0: Mouth opening is 41mm or more, Score 1: Mouth opening is 37 to 40 mm, Score 2: Mouth opening is 33 to 36 mm, Score 3: Mouth opening is 29 to 32 mm, Score 4: Mouth opening is 25 to 28 mm, Score 5: Mouth opening is 21 to 24 mm, Score 6: Mouth opening is 17 to 20 mm, Score 7: Mouth opening is 13 to 16 mm, Score 8:

Mouth opening is 09 to 12 mm, Score 9: Mouth opening is 05 to 08 mm, Score 10: Mouth opening is 0 to 04 mm.

4. Lai DR (1995) divided OSF based on the interincisal distance as follows:

- Group A: >35 mm
- Group B: Between 30 and 35 mm
- Group C: Between 20 and 30 mm
- Group D: <20 mm

6. Ranganathan K et al (2001) divided OSF based on mouth opening as follows:

- Group I: Only symptoms, with no demonstrable restriction of mouth opening.
- Group II: Limited mouth opening 20 mm and above.
- Group III: Mouth opening less than 20 mm.
- Group IV: OSF advanced with limited mouth opening. Precancerous or cancerous changes seen throughout the mucosa.

7. Rajendran R (2003) reported the clinical features of OSF as follows:

- Early OSF: Burning sensation in the mouth. Blisters especially on the palate, ulceration or recurrent generalized inflammation of oral mucosa, excessive salivation, defective gustatory sensation and dryness of mouth.
- Advanced OSF: Blanched and slightly opaque mucosa, fibrous bands in buccal mucosa running in vertical direction. Palate and faucial pillars are the areas first involved. Gradual impairment of tongue movement and difficulty in mouth opening.

9. Chandramani More et al (2011):

- Clinical staging:
- Stage 1 (S1): Stomatitis and/or blanching of oral mucosa.

- Stage 2 (S2): Presence of palpable fibrous bands in buccal mucosa and/or oropharynx, with /without stomatitis.
- Stage 3 (S3): Presence of palpable fibrous bands in buccal mucosa and/or oropharynx, and in any other parts of oral cavity, with/ without stomatitis.
- Stage 4 (S4) as follows:

a. Any one of the above stage along with other potentially malignant disorders, e.g. oral leukoplakia, oral erythroplakia, etc.

b. Any one of the above stage along with oral carcinoma.

- Functional staging:
- M1: Interincisal mouth opening up to or greater than 35 mm.
- M2: Interincisal mouth opening between 25 and 35 mm.
- M3: Interincisal mouth opening between 15 and 25 mm.
- M4: Interincisal mouth opening less than 15 mm.

2. Classifications based on histopathological features of OSF

1. Pindborg JJ and Sirsat SM (1966) were the first to divide OSF depending only on histopathological features alone are as follows:

- Very early stage: Finely fibrillar collagen dispersed with marked edema. Plump young fibroblast containing abundant cytoplasm. Blood vessels are dilated and congested. Inflammatory cells, mainly polymorphonuclear leukocytes with occasional eosinophils are found.
- Early stage: Juxta-epithelial area shows early hyalinization. Collagen still in separate thick bundles. Moderate number of plump young fibroblasts is present. Dilated and congested blood vessels. Inflammatory cells are primarily lymphocytes, eosinophils and occasional plasma cells.

- Moderately advanced stage: Collagen is moderately hyalinized. Thickened collagen bundles are separated by slight residual edema. Fibroblastic response is less marked. Blood vessels are either normal or compressed. Inflammatory exudate consists of lymphocytes and plasma cells.
- Advanced stage: Collagen is completely hyalinized. Smooth sheets with no separate bundles of collagen are seen. Edema is absent. Hyalinized area is devoid of fibroblasts. Blood vessels are completely obliterated or narrowed. Inflammatory cells are lymphocytes and plasma cells.

3. Classification based on clinical and histopathological features

I. Khanna JN and Andrade NN (1995) developed a group classification system for the surgical management of OSF.

- Group I:- Very early cases: Common symptom is burning sensation in the mouth, acute ulceration and recurrent stomatitis and not associated with mouth opening limitation.
- Histology: Fine fibrillar collagen network interspersed with marked edema, blood vessels dilated and congested, large aggregate of plump young fibroblasts present with abundant cytoplasm, inflammatory cells mainly consist of polymorphonuclear leukocytes with few eosinophils. The epithelium is normal.
- Group II: Early cases—Buccal mucosa appears mottled and marble like, widespread sheets of fibrosis palpable, interincisal distance of 26 to 35 mm.
- Histology: Juxta-epithelial hyalinization present, collagen present as thickened but separate bundles, blood vessels dilated and congested, young fibroblasts seen in moderate number, inflammatory cells mainly consist of polymorphonuclear

leukocytes with few eosinophils and occasional plasma cells, flattening or shortening of epithelial rete-pegs evident with varying degree of keratinization.

- Group III: Moderately advanced cases— Trismus, interincisal distance of 15 to 25 mm, buccal mucosa appears pale firmly attached to underlying tissues, atrophy of vermilion border, vertical fibrous bands palpable at the soft palate, pterygomandibular raphe and anterior faucial pillars.
- Histology: Juxta-epithelial hyalinization present, thickened collagen bundles, residual edema, constricted blood vessels, mature fibroblasts with scanty cytoplasm and spindle-shaped nuclei, inflammatory exudates which consists of lymphocytes and plasma cells, epithelium markedly atrophic with loss of rete pegs, muscle fibers seen with thickened and dense collagen fibers.
- Group IVA: Advanced cases—severe trismus, interincisal distance of less than 15 mm, thickened faucial pillars, shrunken uvula, restricted tongue movement, presence of circular band around entire lip and mouth.
- Group IVB: Advanced cases—presence of hyperkeratotic leukoplakia and/or squamous cell carcinoma.
- Histology: Collagen hyalinized smooth sheet, extensive fibrosis, obliterated the mucosal blood vessels, eliminated melanocytes, absent fibroblasts within the hyalinized zones, total loss of epithelial rete pegs, presence of mild to moderate atypia and extensive degeneration of muscle fibers.

CLINICAL SYMPTOMS

Rao AB (1962)[30] noted features like inability to open mouth, intolerance to hot spicy food, inability to protrude their tongue and complained of pain in the ear and swelling and pain around the lower jaw and neck. Also found when the fibrosis reaches

the pharynx, referred pain in the ears and deafness due to occlusion of Eustachian tubes.

Pindborg JJ and Sirsat (1966)[15] in their article noted prodromal symptoms like burning sensation for spicy food, blisters and ulceration or recurrent stomatitis, defective gustatory sensation, excessive salivation and dryness of mouth on OSF patients.

Hayes (1985)[61] reported that the most characteristic feature of OSF is the marked vertical fibrous ridge formation within the cheeks, and board like stiffness of the buccal mucosa leading to trismus and difficulty in blowing cheeks.

Murthi PR et al (1992)[62] elicited the symptoms of oral submucous fibrosis that included trismus, pain and burning sensation on spicy food, altered salivation, change in gustatory sensation, hearing loss due to stenosis of Eustachian tubes, nasal tone to voice, dysphagia to solids and impaired mouth movements.

El Labbon et al (1985)[63] have investigated the muscle changes ultrastructurally in OSF patients and concluded that restricted mouth opening in OSF might be depend upon not only on subepithelial fibrosis, but also on the extent of degeneration of masticatory muscle.

Cox SC and Walker DM (1996)[64] in their review stated that the retromolar areas and the buccal mucosa were commonly involved, followed by soft palate, palatal fauces, uvula, tongue and labial mucosa. The main diagnostic criteria for OSF clinically were the presence of fibrous bands.

Tilakarathne WM et al (2006)[65] reviewed about the disease progression and sequential disabilities of oral functions like restricted mouth opening, inability to blow out a candle or whistle and difficulty in swallowing. They concluded that bands are common at the back of the mouth in mild cases of OSF and as the disease increases in

severity are more likely to be found anteriorly as well.

PREMALIGNANT POTENTIAL:

Paymaster JC (1956)[14] was the first to report the precancerous nature of OSF in 650 patients and described the development of slow growing squamous cell carcinoma in one third of the patients with oral submucous fibrosis.

Pindborg JJ (1972)[66] analyzed 220 biopsies from patients with submucous fibrosis for premalignant changes summarized criteria to support the precancerous nature of this disease as

- Higher prevalence of leukoplakia among OSF patients.
- Higher frequency of epithelial dysplasia.
- Concurrent findings of OSF in oral cancer patients.
- Histopathological diagnosis of oral cancer without clinical suspicion among OSF cases.
- Higher rate of incidence of oral cancer among patients with OSF [3]

Gupta PC et al (1980)[67] reported malignant transformation in 2.3% patients with OSF in a 10 year follow study in Ernakulum District, Kerala.

Pindborg JJ et al (1984)[68] in an evaluation of malignant potential of submucous fibrosis patients after biopsy,12% showed squamous cell carcinoma,26% epithelial dysplasia and 76% atrophic epithelium reinforcing the hypothesis that submucous fibrosis is a precancerous condition.

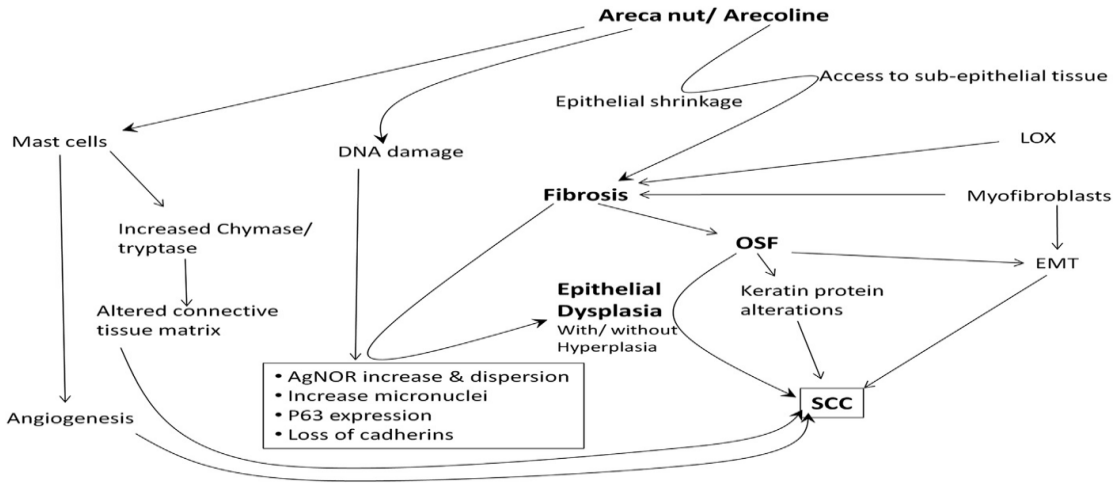
Murti PR et al (1985)[19] reported 7.6% malignant transformation rate in a 17 year follow up study. Oral cancer developed 3-16 years after the diagnosis of submucous fibrosis. The average age at the time of malignant transformation was 64.6 years and age range was 48-81 years.

Murti PR et al (1995)[69] found on their tissue culture experiments using human fibroblasts and suggested that arecanut alkaloids yield powerful carcinogenic nitrosamines which explains the malignant potential of OSF.

Rajendran and Joshy (1998)[70] described submucous fibrosis as a potentially malignant condition of the oral mucosa characterized principally by epithelial atrophy resulting in increased permeability to carcinogen. The disease is initiated in the connective tissue and epithelial changes of atrophy and cytological atypia are secondary.

Recently, the carcinogenicity of areca nut without tobacco was identified, and the second IARC monograph on betel quid has classified areca nut as a 'group one' carcinogen based on epidemiologic and laboratory studies. The strong association of areca nut with OSF, its dose-dependent effects and the confirmation of OSF as a potentially malignant disease leading to oral cancer provided further evidence for this assertion[71]

Ray et al (2016)[72] in the review showed associations observed in studies assessing malignant transformation of oral submucous fibrosis



TREATMENT

The main concern in the condition is the management of trismus and burning sensation of the oral mucosa. A large number of treatment modalities have been tried by both non surgical and surgical approach.

I. Discontinuation of Habit & Counseling[73]

The preventive measures should be in the form of discontinuation of habit, which can be encouraged through education & advocacy. Affected patients should be explained about the disease and its possible malignant potential. Thorough counseling should be given for de addiction.

I1. Medical Management

Steroids[73]

Steroids act as immunosuppressive agents by opposing the action of soluble factors released by sensitized lymphocytes following activation by specific antigens. Steroids also prevent or suppress inflammatory reactions, thereby preventing fibrosis by decreasing fibroblastic proliferation and deposition of collagen. Corticosteroids such as hydrocortisone, dexamethasone, triamcinolone and betamethasone have been used in the treatment of OSF.

Enzymes[73]

Enzymes such as hyaluronidase, collagenase and chymotrypsin are being used for the treatment of OSF. Hyaluronidase breaks down hyaluronic acid, lowers the viscosity of the intercellular cement substances and decreases collagen formation. The effect is short term, but combination with steroids gives longer-term results. Chymotrypsin hydrolyzes ester and peptide bonds, and acts as a proteolytic and anti-inflammatory agent in the treatment of OSF.

Dinesh CG et al (1992)[74] suggested that no one treatment modality is successful in completely eliminating the disease. In patient with grade III and grade IV injectable corticosteroids or hyaluronidase locally is helpful.

Vitamins, Antioxidants and Minerals[73]

The ingredients of betel nut induce excessive reactive oxygen species which damages the cell structures, including lipids and membranes, proteins and nucleic acids. Moreover vitamin deficiency, iron deficiency anemia, and malnutrition can derange the repair of the inflamed oral mucosa, leading to defective healing and the resulting atrophic oral mucosa is more susceptible to the effects of areca nut. Antioxidant vitamins stabilize and deactivate the free radicals before they attack cells. Vitamins A, B complex, C, D, E and minerals like iron, copper and magnesium, when used a standard or adjunct are effective in controlling the signs and symptoms of OSF.

Maher R et al (1997)[75] evaluated the efficacy of multiple micronutrient supplementation including vitamins A, B complex, C, D and E in 117 patients of submucous fibrosis. They noted a significant improvement in symptoms, notably tolerance to spicy foods, burning sensation and signs of mouth opening.

Lycopene is a powerful antioxidant and has a singlet -oxygen-quenching ability twice as high as that of beta-carotene and ten times higher than that of alpha-tocopherol. Patients who received 16 mg of lycopene showed an average increase of 3.4 mm mouth-opening.. The investigators suggested that lycopene is a safe and reliable drug and should be used as a first line of therapy in the initial management of oral submucous fibrosis[76].

Biogenic stimulator[24]

Placentrix is an aqueous extract of human placenta that contains nucleotides, enzymes, vitamins, aminoacids and steroids. It acts by "biogenic stimulation". Such tissues or their extracts, implanted or injected into the body after resistance to pathogenic factors, stimulate the metabolic or regenerative processes, thereby favoring recovery. It has no contraindications and the results obtained are found to be lasting.

Peripheral vasodilator[24]

Pentoxifylline have vasodilating properties, suppresses leukocyte function, stimulates fibrinolysis, inhibits the production of tumor necrosis factor and T and B cell activation. Pentoxifylline 400 mg three times daily, resulted in improvement in mouth opening, tongue protrusion, and relief from perioral fibrotic bands.

Anjum Aara et al (2012)[77] studied clinically to evaluate the efficacy of oral Pentoxifylline 400mg in OSF patients in comparison to intralesional injections of Dexamethasone (4mg/ml) and Hyaluronidase 1500 IU in the management of OSF patients. He concluded that Pentoxifylline can be safer and better alternative in the treatment of oral submucous fibrosis in comparision to dexamethasone.

Combined Therapy[73]

Vitamin D, E & B complex, placental extract, local & systemic corticosteroids & physiotherapy claim a high success rate in oral submucous fibrosis management.

Other Drugs

Interferon-Gamma[73]

IFN-gamma is a known anti-fibrotic cytokine. Patients treated with an intra-lesional injection of IFN-gamma experienced improvement of symptoms.

Immune milk

Tai et al (2001)[78] studied the Immune milk treatment in OSF patients. The immune milk contains an anti-inflammatory component that may suppress the inflammatory reaction and modulate cytokine production. Symptomatic relief in patients may be partially attributed to the micronutrients contained in the immune milk powder.

Ayurvedic therapy[73]

Administration of turmeric powder offers protection against benzopyrene induced increase in micronuclei in circulating lymphocytes and it is an excellent scavenger of free radical in vitro. Turmeric oil & turmeric oleoresin both act synergistically in vivo to offer protection against DNA damage.

Deepa Das et al[79] found that turmeric dispensed in the form of cucurmin and turmeric oil was effective in the treatment of OSF which was evident by the positive changes observed in the histopathological examination after treatment along with the significant improvement in clinical signs and symptoms.

Oral Physiotherapy[73]

Muscle stretching exercises for the mouth may be helpful to prevent further limitation of mouth movements. This includes forceful mouth opening with the help of sticks, ballooning of mouth, hot water gargling. This is thought to put pressure on fibrous bands. Forceful mouth opening have been tried with mouth gag & acrylic surgical screw.

Cox and Zoellner (2009)[80] advocated five times daily physiotherapy by inter-positioning tongue spatulas between teeth and adding a new spatula every 5–10 days for 4 months and observed improved oral opening.

Surgical Treatment[73]

Surgical treatment is indicated in patients with severe trismus and/or biopsy results revealing dysplastic or neoplastic changes. It includes simple excision of the fibrous bands, with major limitation being contracture of the tissue and exacerbation of the condition.LASER - CO2 laser surgery offers advantage in alleviating the functional restriction.

Cryosurgery[73]

It is the method of local destruction of tissue by freezing it in situ. Extreme cold is produced by liquid nitrogen or argon gas to destroy abnormal tissue. Liquid sprays are better suited for mucosal lesion.

HOMOCYSTEINE

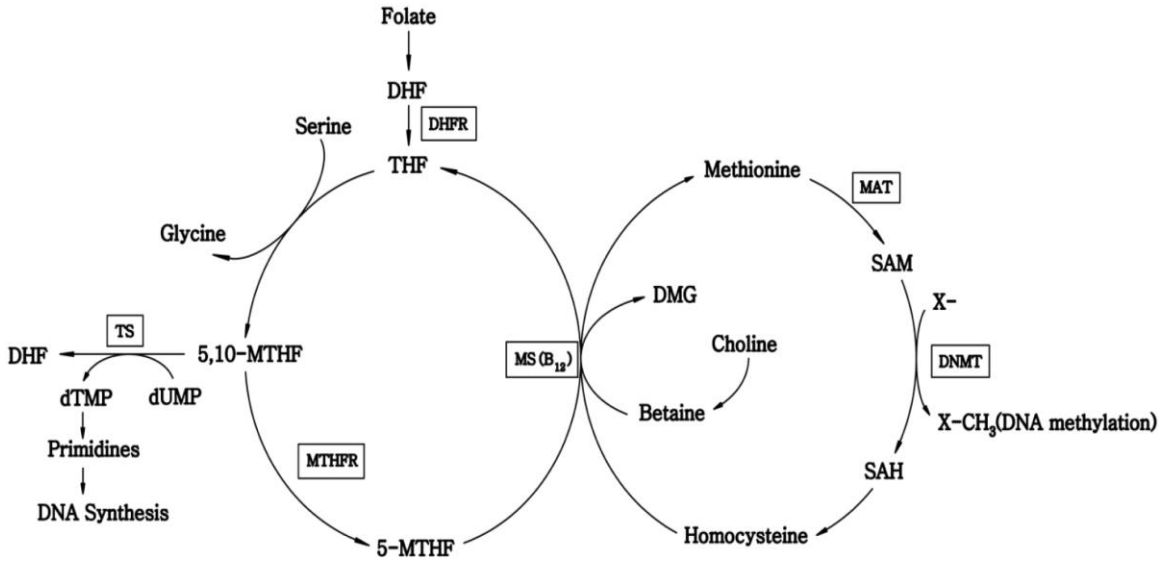
Homocysteine is sulfur containing non-protein amino acid, derived from methionine an essential amino acid. It was discovered as early as in 1930s by Du Vigneaud, an American biochemist who named it Hcy, as its structure was found to be similar to cysteine with an extra carbon atom[1].Hcy exists in different forms, namely Hcy homocystine, Hcy dimer, Hcy thiolactone and Hcy adducts forming the total Hcy[9].

Hcy plasma levels can be measured by enzyme assays, enzyme-linked immunosorbent assay (ELISA) and high performance liquid chromatography (HPLC)[2]. Usually the tHcy levels are measured. The normal plasma tHcy is about 5-15µmol/L. Hyperhomocysteinemias are graded as follows: Mild 15-30 µmol/L, Moderate 30-100µmol/L, Severe >100µmol/L. Hcy levels above 50 µmol/l is thought to be a risk factor for recurrent heart attacks and a value between 150 and 200 µmol/L may cause ischemic stroke. Levels >300 µmol/L may induce mental deficiency[9].

METABOLIC PATHWAY OF HOMOCYSTEINE

The only source of Hcy in the body is methionine. Hcy can enter into 2 pathways. One is the remethylation pathway that depends on vitamin B12 to form methionine and the other the trans-sulfuration pathway that depends on vitamin B6 to form cysteine. Under normal conditions, most of the Hcy is remethylated back to methionine or converted into cysteine by the trans-sulfuration pathway. Three B complex vitamins are involved in Hcy metabolism viz. B6, folate or B9, and B12. Deficiency of these vitamins and enzymes involved in the metabolism increases plasma Hcy levels[9].

The elevated serum concentration of total homocysteine (Hcy), a well-known cardiovascular risk factor, and consequent deficiency of folate, vitamin B12, or vitamin B6, or genetic polymorphisms involves the transfer of one-carbon groups. The mechanism has been considered critical for Hcy metabolism in carcinogenesis in terms of DNA synthesis, repair and methylation[81].



Flow diagram of Homocysteine Metabolism

FACTORS AFFECTING HOMOCYSTEINE

Hcy levels in general are found to be higher in men, postmenopausal women, in those with increased muscle mass and in the elderly. Smoking, excess alcohol (chronic alcoholics, intoxication) and coffee consumption (>5 cups/day), Vegans (those who do not consume eggs and milk) are known to cause HHcy. All these factors may affect the remethylation pathway thus raising Hcy levels. Antiepileptics like phenytoin, phenobarbitone and valproate, others like metformin, raise plasma Hcy levels.[9].

Martha Savaria et al (2000)[82] showed that total serum HCY concentrations of premenopausal women of all ages were statistically significantly lower than those of comparably aged men. Moreover, they found higher plasma homocysteine concentrations in postmenopausal versus premenopausal women.

Chao MC et al (2013)[83] reported that subjects with elevated homocysteine levels were more likely to be betel nut chewers compared to subjects with normal homocysteine levels.

MECHANISM OF HYPERHOMOCYSTEINEMIA INDUCED DAMAGE[9]

The mechanism of action of Hcy in causing ill health and disease appears to be complex and not clearly known. The various manifestations of HHcy are explained on the basis of oxidative damage and protein homocysteinylation.

1. Oxidative Damage

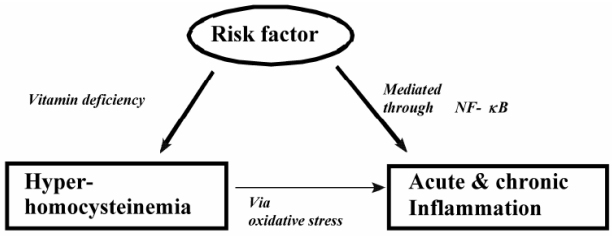
Hcy (with –SH group) continuously gets oxidized resulting in homocysteine (S–S group) and homocysteinylated proteins. This leads to the formation of reactive oxygen species (ROS) such as super oxide, hydrogen peroxide and hydroxyl free radicals leading to damaging effects on biomolecules.

2. Protein Homocysteinylation

Hcy thiolactone is highly reactive and acylate free amino groups of different proteins under physiological conditions. The process referred as N–homocysteinylation. The degree of protein homocysteinylation increases with increased plasma tHcy and largely contributes to manifestations of Hcy toxicity. Homocysteinylated proteins may become inactive or result in modified proteins that are detrimental to health. Homocysteinylation causes immune activation, autoimmune inflammatory response, cellular toxicity and cell death, and enhanced protein degradation.

ASSOCIATION WITH MARKERS OF ACUTE AND CHRONIC INFLAMMATION[10]

The oxidative stress derived from hyperhomocysteinemia will again induce acute and chronic inflammation via the regulation of NF-κB transcription factor. Investigators have failed to reduce the level of inflammation markers when they had successfully lowered the level of circulating homocysteine with the administration of vitamins. Thus concluded that long as the risk factor (s) is not removed, it would continue to generate acute and chronic inflammation regardless whether of deficiency or not.



Hofmann et al (2001)[84] proposed that Hyperhomocysteinemia has often been associated with inflammation and Homocysteine has been identified as a contributor to four fundamental mechanisms of disease: thrombosis oxidant stress, apoptosis and cellular proliferation.

Papatheodorou et al (2007)[85] proposed that homocysteine (Hcy) induces inflammation, possibly by enhancing oxidative stress and subsequent nuclear factor kappa B (NfκB) activation.

MEASURES OF PREVENTION

Kim and Pae (1996)[86] showed that the toxic influence of homocysteine to the endothelium can be blocked by antioxidant enzyme supplementation.

Uppala et al (2012)[9] summarized the measures on prevention of hyperhomocysteine such as consuming healthy nutritious foods rich in folate, cobalamin and pyridoxine. Lifestyle changes like abstaining from smoking and alcohol and reducing coffee consumption. Vitamin supplements like Folic acid B9, B12 to lower Hcy levels

HOMOCYSTEINE AND SYSTEMIC DISEASE[9]

Hcy induces oxidative stress resulting in increased production of 3-hydroxy 3-methyl glutaryl coenzyme A (HMG CoA) in the endothelial cell that leads to increased cholesterol production.. An increase by 1 µmol/l of plasma Hcy increases the risk of cardiovascular disease by 10%.

HHcy is implicated in ocular vascular disease. It is associated with retinal artery occlusion, diabetic retinopathy, age related macular degeneration, choroidal neovascularization, and glaucoma.

It causes cerebral ischemia leading to cerebrovascular accidents. Methyl malonic acid is neurotoxic and the symptoms range from mild irritability, mood swings, and forgetfulness to depression and dementia. Alzheimer's dementia is associated with amyloid precursor protein and neurofibrillary tangles, which are due to improper gene expression caused by deficient DNA methylation.

In diabetes mellitus (DM), HHcy associated microvascular complications accelerates atherosclerosis by endothelial damage induced by oxidative stress. It was also found that HHcy is associated with insulin resistance.

Hyperhomocysteinemia is seen in end stage renal disease. HHcy causes endothelial dysfunction and damage to glomerular endothelial cell through oxidant stress.

HHcy is also associated with an increased risk of fractures. It is hypothesized that increase in plasma Hcy levels prevent the formation of collagen cross links. The bone matrix is not proper and this leads to fragile bones. The enzyme affected is lysyl oxidase and its function is impaired due to homocysteinylation.

Forges et al (2007)[87] summarized the damage to the reproductive system caused by HHcy in males and females. In males, the increased plasma Hcy level may lead to infertility. In females, Hyperhomocysteinemia is also associated with polycystic ovarian disease, recurrent pregnancy loss, defective ovulation, improper embryonic cleavage and poor implantation.

HOMOCYSTEINE AND ORAL DISEASES

Gonul M et al (2009)[88] conducted a study in 45 Bechets disease and 47 recurrent aphthous stomatitis patients to compare the homocysteine levels and observed significantly higher homocysteine levels in BD compared but homocysteine levels in RAS patients were not significantly different from those of healthy controls.

Sun A et al (2012)[5] conducted a study in 176 atrophic glossitis patients and found significant association of deficiency of hemoglobin, iron and vitamin B12, abnormally high blood homocysteine level, and serum GPCA positivity with AG.

Sun A et al (2012)[89] conducted a study in 91 atrophic glossitis patients to evaluate whether supplementation of different vitamins and iron could reduce the serum homocysteine levels and found significant reduction in homocysteine levels.

Lin HP et al (2013)[4] conducted a study in 399 Burning Mouth Syndrome patients to evaluate association of hematinic deficiencies and high blood homocysteine levels with burning mouth syndrome and found 89 (22.3%) had abnormally higher blood homocysteine level.

Sun A et al (2013)[90] conducted a study in 399 patients with burning mouth syndrome. They were treated with vitamin supplements and found complete remission of oral symptoms with significant reduction in serum homocysteine levels.

Chen HM et al (2015)[3] conducted a study in 352 Oral Lichen Planus patients to assess association of the deficiencies of hemoglobin (Hb), iron, vitamin B12, and folic acid and high blood homocysteine level and found abnormally elevated blood homocysteine level than healthy control participants and concluded that there is a close relation of high blood homocysteine level to severity of OLP.

Sun A et al (2015)[6] conducted a study in 273 RAS patients and demonstrated significantly greater frequencies of Hb, serum iron, vitamin B12, and folic acid deficiencies and high serum homocysteine level (7.7%)than healthy control subjects.

Chang JYF et al (2015)[91] conducted a study in 149 oral mucosal disease patients with both vitamin B12 and iron deficiencies to evaluate anemic status, MCV, serum and found 44 (29.5%) had abnormally higher blood homocysteine level.

Chang JYF et al (2016)[92] in the study assessed hematinic deficiencies and anemia statuses in 92 antigastric parietal cell antibody positive Erosive Oral Lichen

Planus patients with Desquamative Gingivitis and had found 37 (40.2%) abnormally high blood homocysteine level than healthy control individuals.

Wu YC et al (2016)[93] conducted a study in 160 RAS with atrophic glossitis patients and 195 RAS without atrophic glossitis patients and found that both had significantly greater frequencies of Hb, serum iron, vitamin B12, and folic acid deficiencies and of high serum homocysteine level than healthy control subjects.

HOMOCYSTEINE AND CARCINOMA

Refsum et al (1991)[94] conducted a study in 12 children with acute lymphoblastic leukemia found increased tHcy in seven children even before the beginning of any treatment and elevated circulating tHcy fell drastically within a few days after treatment with cytotoxic drugs. Thus the circulating tHcy reflected the total burden of malignant circulating leukemic cells.

Wu LL and Wu JT (2002)[95] in the study found elevated circulating total homocysteine (tHcy) in cancer patients even though they were not treated with antifolate drugs and the change coincided with the concentration of tumor markers in patients undergoing treatment Thus tHcy may be used as a more accurate tumor marker for monitoring cancer patients during treatment and hyperhomocysteinemia as a risk factor for carcinogenesis.

Sun CF et al (2002)[96] employed tissue cultures of specimens from breast, ovarian and pancreatic carcinoma to compare both the Hcy released and production of tumor markers between tumor and normal cell lines and detected much higher concentrations of Hcy released by the tumor cells. Serum Hcy may be a potentially useful tumor marker to monitor tumor activity.

Almadori et al (2002)[97]analyzed serum concentrations of two metabolites ,folate and homocysteine, in 42 patients affected by head and neck squamous cell carcinoma (HNSCC) in comparison with control groups. The results were statistically significant. The differences in serum levels of folate and homocysteine might arise from tumor development and consequent metabolic alterations or might precede and promote tumor progression.

Almadori (2005)[98] In the study on 144 untreated patients with HNSCC and 40 untreated patients with laryngeal leukoplakia compared with control group. The found that serum homocysteine levels in patients with HNSCC were significantly higher compared with laryngeal leukoplakia that did not differ significantly from the controls. These exclude the role for a high homocysteine serum level both as a risk marker and marker of neoplastic progression.

Eleftheriadou et al (2006)[8] measured the serum levels of folate and homocysteine in 150 patients with histologically proven SCCHN before any treatment and in 150 healthy volunteers. The study indicated a positive correlation between hyperhomocysteinemia and hypofolatemia and the presence of SCCHN.

Ozkan et al (2007)[99] conducted a study in newly diagnosed 37 untreated lung cancer patients and compared with healthy controls found there were significantly higher (t-Hcy) and lower folate levels in the advanced-stage group compared with controls.

Gorgulu et al (2010)[100] in the study to evaluate the role of serum homocysteine, folate, and vitamin B12 levels in the pathogenesis of laryngeal squamous cell cancer (LSCC) by measuring serum levels in 60 consecutive untreated patients with LSCC and 60 controls. There were no significant differences in the homocysteine levels between these three groups.

Ierardi et al (2013)[11] evaluated the serum Homocysteine and Cysteine as a biomarkers of disease progression in breast tumor A significant difference was observed between pre- and post-treatment levels of Homocysteine and Cysteine in advanced tumors, suggesting a prognostic role in patients with poor clinical characteristics. The research suggested that Hcy might be used as a prognostic biomarker for breast cancer.

Zhang et al (2015)[81] in the meta-Analysis of 83 Case-Control Studies Involving 35,758 Individuals covered more than 14 types of cancer and concluded that High level of Hcy but low level of folate was associated with risk of cancer overall, with little effect by type of cancer or ethnicity.

Fanid et al (2015)[101] in the prospective study, The European Prospective Investigation into Cancer and Nutrition recruited 385,747 participants from 10 countries who donated a blood sample. The study included 516 cancer cases of the head and neck and esophagus and 516 individually matched controls. Plasma levels of vitamins B2, B6, B9, B12, and methionine and homocysteine were measured. The analysis revealead an association between elevated levels of the amino acid homocysteine and increased risk of squamous cell carcinoma of the head and neck

HOMOCYSTEINE IN ORAL SUBMUCOUS FIBROSIS

Bais et al (2013)[102] conducted a cross-sectional study in a newly diagnosed 50 OSF patients in a tertiary care hospital. The homocysteine level was found to be higher among the patients of stage IV (31.02±6.33) than stage III (26.98±8.67) and II (25.47±7.72). There was no significant difference in the level of homocysteine by gender and clinical staging.

Narang D et al (2014)[103] in a study to estimate serum homocysteine level in a newly diagnosed 50 OSF patients found an increased level in all patients irrespective of

gender and age but no statistically significant co-relation was found among clinical stages and pathological grading. The study concludes that chronic inflammation in OSF leads to hyperhomocysteinemia which could be used to assess the level of severity of disease and may be used as prognosticator marker of therapeutic response for treatment of the disease.

Jaganath et al (2016)[26] conducted a study in 50 patients, comprising of 20 cases each of OSF and OSCC and 10 healthy controls. The plasma homocysteine levels were elevated in OSF and OSCC patients as compared to healthy controls. Conversely levels in OSCC patients were lower compared to OSF patients. Although statistically insignificant, the altered plasma homocysteine level played a vital role in the pathogenesis of these conditions as it brings about oxidative DNA damage, initiating carcinogenesis. The supplementation with vitamins could act as chemopreventive agents in combating hyperhomocysteinemia, arresting the disease progression in OSF and aiding in and treatment of OSCC.

MATERIALS AND METHODS

The study was conducted after getting approval from the Institutional Ethical Committee.

Study Centre:

1. Department of Oral Medicine and Radiology,
   Tamil Nadu Government Dental College and Hospital,
   Chennai - 600003.
2. Lister Metropolis Healthcare Limited,
   Nungambakkam,
   Chennai - 600034.

Study Population:

The study population was selected from the outpatient section of Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai. Participants who were satisfying the following inclusion and exclusion criteria were included in the study.

Inclusion criteria for cases:

- Patient with age group of 20 to 50 years and of both gender.
- Newly diagnosed patients who satisfy the characteristic clinical features of OSF.
- Patients who are not under any medication for the same.
- Patients willing to participate in the study.

Inclusion criteria for controls:

- Patient with age group of 20 to 50 years and of both gender.
- Patients willing to participate in the study**.**

Exclusion criteria for cases:

- Patients with chronic systemic diseases like Cardiac, Cerebrovascular, Respiratory, Renal, Hepatic, Gastrointestinal disorders, Collagen disorders, Infectious diseases, Bleeding disorders and Diabetes Mellitus and Cancer.
- Patients who are Pregnant (or suspected to be conceived) and Lactation.
- Patients under medication for systemic illness (anticonvulsant, antifolate, oral hypoglycemic drugs).
- Patients who are contraindicated for steroids.
- Patient with oral submucous fibrosis who show malignant transformation.
- Patients with oral submucous fibrosis who are indicated for surgical management.
- Patients suffering from known systemic nutritional deficiencies.
- Patients not willing to participate in the study.

Exclusion criteria for controls:

- Patients with history of habits.
- Patients with oral mucosal disease.
- Patients with known systemic illness or under any medication.
- Patients who are Pregnant (or suspected to be conceived) and Lactation.
- Patients not willing to participate in the study.

Sampling Procedure:

Convenient Sampling

Sample Size:

Sample size: 37 **[**Healthy Controls - 7, OSF Patients - 30]

Study Design: Prospective Study

METHODOLOGY:

Based on inclusion and exclusion criteria, 37 study subjects comprising of 30 OSF patients and 7 healthy controls were included in the study. All the participants were explained about the need and design of the study, the need for undergoing a thorough clinical examination, routine investigations and Serum Homocysteine evaluation as a part of the study. The OSF patients were explained about the drug therapy and their possible adverse effects. Only those subjects who gave a signed informed consent on an institutionally approved document were included in the study. Patients were numbered serially as they entered the study.

Patients were made to sit comfortably on a dental chair. A detailed case history of the patient with emphasis on their habits and a brief medical history was taken to rule out any possible systemic illness. Then thorough clinical examination was carried out wearing sterile hand gloves and mouth mask under artificial illumination. A clinical diagnosis of OSF was made based on positive history of chewing arecanut or one of its commercial preparation, difficulty in mouth opening, burning sensation on eating spicy foods and clinical findings of presence of restricted mouth opening, changes in oral mucous membrane such as blanching of oral mucosa and presence of palpable fibrous bands. The details were recorded on a structured proforma designed for the study.

The OSF patients were then graded clinically, according to **Khanna JN, Andrade NN (1995),**

Grade I: Very early stage

Normal mouth opening

Burning sensation in the mouth

Acute ulceration and recurrent stomatitis

Grade II*:* Early stage

Interincisal distance of 26 to 35 mm

Buccal mucosa appears mottled and marble like

Widespread sheets of fibrosis palpable and red erythematous patches

Grade III*:* Moderately advanced stage

Interincisal distance of 15 to 25 mm

Buccal mucosa appears pale firmly attached to underlying tissues

Atrophy of vermilion border

Vertical fibrous bands palpable at the soft palate, pterygomandibular raphe and anterior faucial pillars

Grade IVA*:* Advanced stage

Severe trismus

Interincisal distance of less than 15 mm

Restricted tongue movement

Presence of circular band around entire lip and mouth

Thickened faucial pillars, shrunken uvula

Grade IV B: Advanced with premalignant and malignant changes

Oral submucous fibrosis and Leukoplakia

Oral submucous fibrosis and Squamous cell carcinoma

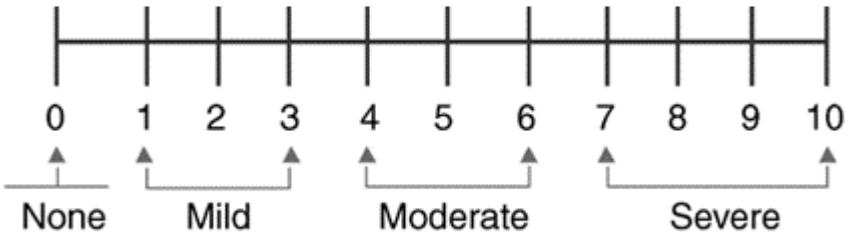
All the participants in control group and OSF group were subjected to Complete Haemogram, urine investigation and Homocysteine evaluation.Before the drug administration, 30 OSF Patients were subjected to Tobacco Cessation Counselling

(TCC) in the institution and TCC were continued during the progress of the study. Oral prophylaxis was also carried out in all these patients before Homocysteine evaluation.

COLLECTION OF DATA

Clinical parameters included in the study to evaluate the effectiveness of the drugs were burning sensation and mouth opening.

The intensity of burning sensation was determined using a Visual Analogue Scale (VAS) of 0-10 with 10 mm division, where 0 was no burning sensation and 10 was worst possible burning sensation. The patients were asked to mark VAS at a point which best represented their level of symptoms.



The interincisal mouth opening was measured using divider and scale from the mesio-incisal angle of upper central incisor to the mesio-incisal angle of lower central incisor and recorded in centimetres. If the corresponding teeth were not present contra lateral teeth or adjacent teeth will be considered.

HOMOCYSTEINE ESTIMATION:

Blood Sample Collection:

All the study participants were advised overnight fasting to avoid any dietary influence and blood samples were collected during the morning hours. Patients were made to sit comfortably on a chair. Tourniquet was then applied above the Antecubital

fossa. Venipuncture site located, Median cubital vein was palpated and the area cleansed using alcohol wipes. Venipuncture performed using a 23 gauge needle and 5ml of blood was withdrawn. Tourniquet was released and the needle was withdrawn. Finger pressure was applied over the punctured site using cotton for 5 minutes.

Blood from the syringe was then transferred to the SST vacutainer tube and gently inverted for 5 times to activate clotting. Needle in syringe was destroyed using needle destroyer and disposed in a puncture proof container. Patient was checked for any bleeding from the site and adhesive bandage was applied.

Blood sample was labeled and placed in a zip lock bag. Then transferred to the transportation box and placed in between the pre-frozen gel pack. The lid was closed and transported immediately to the laboratory. The processing and analysis was performed at the Lister Metropolis Laboratory [ISO 15189: 2007 certified lab], Chennai.

Sample Processing:

Blood was allowed to clot for 20 minutes and centrifuged under 3500 rpm for 10 minutes. An inert gel at the bottom of the vacutainer tube moves to the serum/clot interface during centrifugation, thus providing a barrier between the serum and the clotted blood. The barrier gel in SST tubes facilitates rapid separation of serum from cellular constituents of blood and thus reduces hemolysis and reduced need for aliquot tubes. The test tube was then removed from the centrifuge machine.

Estimation procedure:

Homocysteine was measured in the serum samples by a fully automated ARCHITECT Homocysteine assay (ARCHITECT *i* system, ABBOTT Laboratories USA). The ARCHITECT Homocysteine assay uses Chemiluminescent Microparticle

Immunoassay (CMIA) technology for the quantitative determination of serum Homocysteine.

Before loading the Reagent Kit on the system, the microparticle bottle is inverted 30 times to resuspend microparticles that have settled down and septum is placed on all reagent bottles. The ARCHITECT Homocysteine Reagent Kit is then loaded on the ARCHITECT *i* System. Calibration and control tests are ordered. Then serum samples are loaded and procedure initiated.

Principle of the procedure is that the bound or dimerised homocysteine (oxidized form) is reduced by dithiothreitol (DTT) to free homocysteine, which is then converted to S-adenosyl homocysteine (SAH) by the action of the recombinant enzyme S-adenosyl homocysteine hydrolase (rSAHHase) in the presence of excess adenosine. The SAH then competes with acridinium-labeled S-adenosyl cysteine for particle-bound monoclonal antibody. Following a wash stage and magnetic separation, pre-trigger and trigger solutions are added to the reaction mixture and the resulting chemiluminescence is measured as relative light units (RLUs). An indirect relationship exists between the amount of homocysteine in the sample and the RLUs detected by the ARCHITECT *i* System optics. Results are obtained by using a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method by generating a calibration curve. The values are then recorded.

Reference range for Male was 5.46 - 16.20 and for Female was 4.44 - 13.56. and expressed in µmol/L.

METHOD OF DRUG ADMINISTRATION:

After Homocysteine evaluation, Grade I OSF patients were given supplemental medication of Capsule antioxidant twice daily, Tablet Multivitamin once daily and Tablet vitamin B complex twice daily for 6 weeks whereas patients in Grade II, Grade III and Grade IV were administered intralesional injection of 0.5ml of local anaesthesia with 2ml of dexamethasone twice weekly and supplemental medication for 6 weeks.

Composition of Capsule Antioxidant which was used as a supplemental medication was - Alpha lipoic acid 50 mg + Beta-carotene 10 mg+ Elemental copper 1 mg + Elemental selenium 75 mcg + Lycopene 5 mg + Vitamin E 10 IU +Zinc sulphate 27.45 mg, Tablet Multivitamin – vitamin A 2500IU, vitamin D3 200IU, vitamin B1 2mg, vitamin B2 2mg, vitamin B6 0.5mg, vitamin C 50 mg, folic acid 0.2mg, calcium pantothenate 1mg, niacinamide 25mg and Tablet Vitamin B complex - vitamin B1 2mg, vitamin B2 2mg, vitamin B6 0.5mg, calcium pantothenate 1mg, niacinamide 25mg.

Patient in Grade I were recalled once in a week whereas patients in Grade II, Grade III and Grade IV were recalled twice in a week and evaluated for improvement in signs and symptoms such as burning sensation and mouth opening. Final changes in burning sensation and mouth opening were recorded after the complete course of treatment.

POST OPERATIVE HOMOCYSTEINE EVALUATION:

After the course of the treatment, all the patients were subjected to post operative Homocysteine evaluation similar to pre-operative evaluation and the values are recorded. All the values were statistically analysed and the results were drawn.

ARMAMENTARIUM

Examination of the patient:

- Electrically operated dental chair
- Patient's apron
- Disposable mouth mask
- Disposable latex examination gloves
- Stainless steel kidney trays
- Mouth mirror
- Stainless steel probe
- Tweezer
- Divider and Metallic scale

Blood sample collection:

- A pair of disposable gloves
- Tourniquet
- Sterile Alcohol wipes
- Adhesive bandages
- Sterile Cotton rolls
- Sterile 5 ml disposable syringe
- 23 gauge needle of 1" length
- 3.5ml BD SST II Plus Vacutainer tube
- Gel pack
- Transportation box

Homocysteine Evaluation:

- Centrifuging machine used at 3500 rpm
- ARCHITECT *i* SR 2000 Equipment
- 1L71 ARCHITECT Homocysteine Reagent Kit
- 1L71-01 ARCHITECT Homocysteine Calibrators
- 1L71-10 ARCHITECT Homocysteine Controls

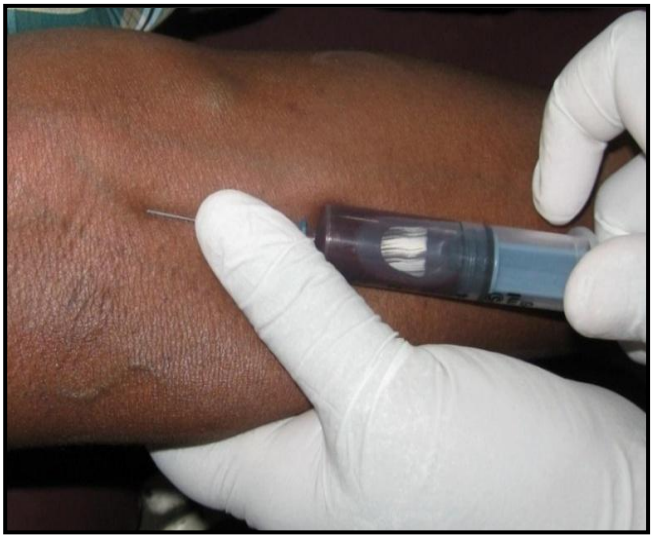
Drug administration:

- Sterile 2 ml disposable syringe
- Topical local anesthetic gel
- Injection Local anaesthesia
- Injection dexamethasone 2ml vial
- Capsule Antioxidant
- Tablet Multivitamin
- Tablet Vitamin B Complex

FIGURE 1: DIAGNOSTIC INSTRUMENT

FIGURE 2: INSTRUMENTS FOR COLLECTION OF BLOOD

FIGURE 3: COLLECTION OF BLOOD SAMPLE



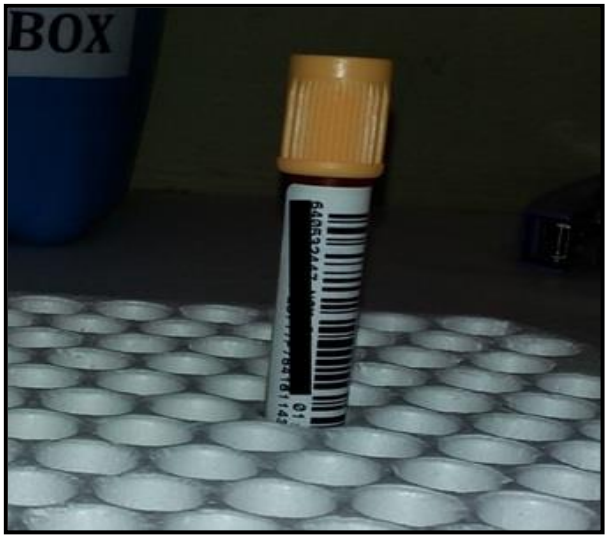


FIGURE 4: TRANSPORTATION KIT

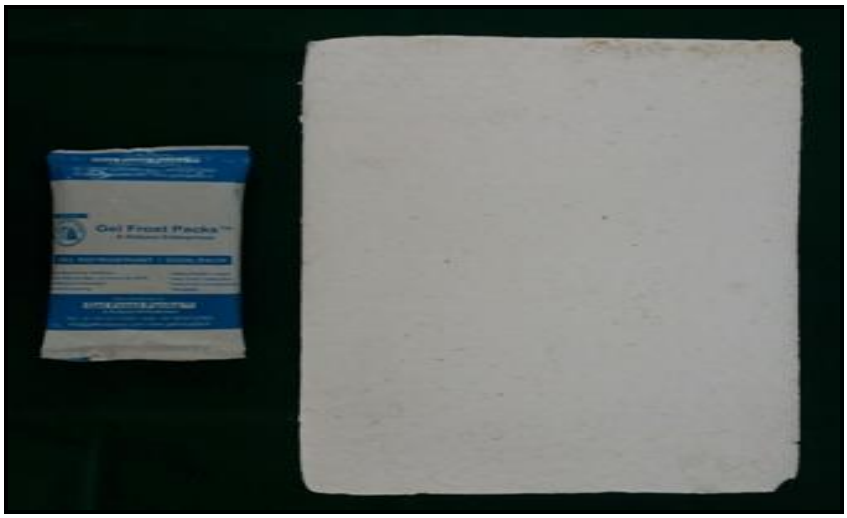


FIGURE 5: CENTRIFUGE MACHINE

FIGURE 6: SERUM SAMPLE

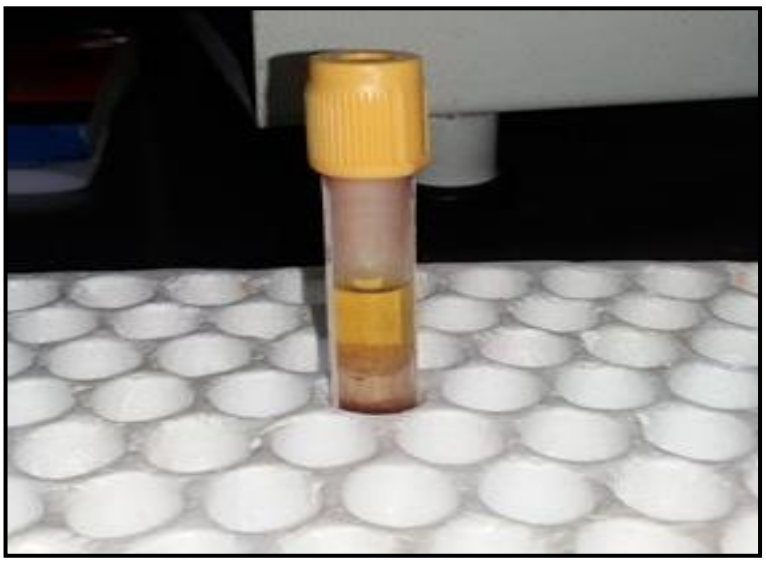


FIGURE 7: REAGENTS FOR HOMOCYSTEINE ESTIMATION

FIGURE 8: ARCHITECT *i* 2000 SR EQUIPMENT

FIGURE 9: BLANCHING OF BUCCAL MUCOSA

FIGURE 10: BLANCHING OF LABIAL MUCOSA

FIGURE 11 : BLANCHING OF SOFT PALATE

FIGURE12: MOUTH OPENING MEASUREMENT

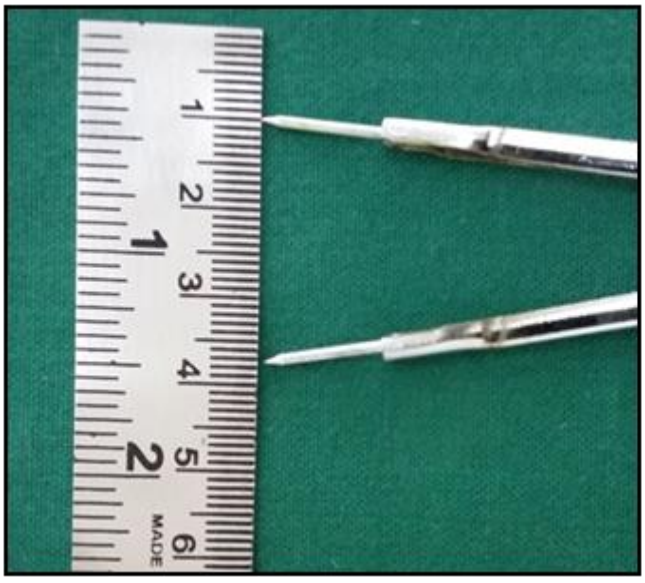


FIGURE 13: DRUGS USED IN THE STUDY

FIGURE 14: INTRALESIONAL INJECTION THERAPY

MASTER CHART

OSF Group:

S. No	Age	Sex	Grade	Chewing Duration (per year)	Chewing Frequency (per day)	Burning Sensation		Mouth Opening (mm)		Homocysteine (µmol/L)	
						Pre op	Post op	Pre op	Post op	Pre op	Post op
1	31	M	I	4	3	3	0	42	46	12.6	10.3
2	37	M	I	7	4	5	0	45	48	11.3	9.8
3	33	F	I	3	2	6	0	39	42	7.2	6.4
4	41	M	I	9	3	4	0	36	40	10.2	7.4
5	26	M	I	3	4	8	0	41	45	12.3	7.1
6	28	F	I	2	2	7	0	42	44	8.2	7.65
7	33	M	I	8	3	7	0	37	41	13.0	8.6
8	30	M	II	6	2	4	0	30	33	15.7	10.2
9	39	M	II	10	4	7	0	26	30	19.2	17.1
10	22	M	II	2	3	3	0	32	37	14.7	10.95
11	29	M	II	5	2	6	0	35	38	16.8	13.5
12	25	M	II	7	3	5	0	29	33	15.3	12.0
13	41	M	II	20	3	10	0	28	31	21.0	19.7
14	20	M	II	5	4	4	0	32	38	14.1	12.3
15	25	M	II	2	5	6	0	31	36	18.7	16.1
16	40	M	III	18	3	7	0	19	23	21.6	19.3
17	39	M	III	13	2	6	0	24	29	20.4	15.7
18	24	M	III	7	8	8	1	18	21	28.3	21.2
19	22	M	III	4	5	6	0	25	29	17.3	14.5
20	27	M	III	6	3	7	0	16	19	21.2	13.4
21	33	F	III	9	2	4	0	24	30	11.4	9.6
22	25	M	III	7	5	8	0	20	24	23.1	12.7
23	39	M	III	5	6	5	0	19	22	20.2	16.4
24	32	M	IV	20	7	10	2	11	13	37.6	28.2
25	20	M	IV	6	10	8	0	14	19	40.9	23.1
26	39	M	IV	11	15	10	3	10	13	50.8	31.6
27	21	M	IV	5	10	8	0	12	15	45.7	24.4
28	29	M	IV	7	8	7	0	14	18	38.4	20.9
29	48	F	IV	13	3	7	1	13	17	11.4	7.99
30	27	M	IV	6	7	6	0	11	15	21.4	14.8

Control Group:

S.No	Age	Sex	Grade	Chewing Duration (per year)	Chewing Frequency (per day)	Burning Sensation	Mouth Opening (mm)	Homocysteine (µmol/L)
1.	25	M	NA	NA	NA	0	53	7.6
2.	48	M	NA	NA	NA	0	51	10.1
3.	34	F	NA	NA	NA	0	48	6.1
4.	31	M	NA	NA	NA	0	52	7.9
5.	29	M	NA	NA	NA	0	55	7.3
6.	35	M	NA	NA	NA	0	51	8.6
7.	42	M	NA	NA	NA	0	48	11.2

NA – Not Applicable

STATISTICAL ANALYSIS

The statistical analysis was done using the computer software program SPSS version 22.

The Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results reveal that the variables follow Normal distribution. Therefore to analyse the data parametric methods are applied. To compare the mean values between groups one way ANOVA is applied followed by Tukey's HSD post hoc tests for multiple pair wise comparisons.

To compare pre and post intervention mean values paired t-test is applied. To compare proportions between study and control groups Chi-Square test is applied, if any expected cell frequency is less than five, then Fisher's exact test is used. Pearson correlation coefficient is calculated to measure the linear relationship between variables.

In the present study, $p < 0.05$ was considered as the level of significance.

RESULTS

A total of 37 study participants comprising of 30 OSF patients and 7 healthy controls were included in the study. OSF patients were graded clinically into 4 grades. They were Grade I (n = 7), Grade II (n = 8), Grade III (n = 8) and Grade IV (n = 7).

AGE DISTRIBUTION

The age range included in the study was 20-50 years with the mean age of 31.59 ± 7.588. The mean age being 34.86 ± 7.86 in control group, 32.71 ± 5.12 in Grade I, 28.88 ± 7.624 in Grade II, 31.13 ± 7.51 in Grade III, 30.86 ± 9.95 in Grade IV. The maximum number of OSF was in the age group of 20 – 30 years (n=15) and minimum were in 40 -50 (n = 3) years of age. [TABLE 1, 2]

GENDER DISTRIBUTION

Out of 37 subjects enrolled in the study 32 (86.5 %) were males and 5 (13.5) were females. In comparision of proportion of study participants, in control group, 6 (85.7 %) were males and 1 (14.3 %) were females whereas in OSF group, in Grade I, 5 (71.4 %) were males and 2 (28.6 %) were females, in Grade II, 8 (100 %) were males and 0 (0.0 %) were females, in Grade III 7 (85.7 %) were males and 1 (12.5 %) were females, in Grade IV 6 (85.7 %) were males and 1 (14.3 %) were females. Among the OSF patients, a male predilection (n = 28) was observed. [TABLE 3]

HABITS:

In the present study, all the OSF patients gave a positive history of arecanut chewing in the form of commercial preparation such as pan masala or mawa. The most common form of arecanut used was found to be mawa with 80 % (n=24).

The frequency of chews per day in OSF patients varied between 2 and 15 per day. The mean frequency of chews being 5.14 ± 2.795 per day in Grade I, 6.50 ± 4.30 per day in Grade II, 8.13 ± 3.64 per day in Grade III and 9.17 ± 5.40 per day in Grade IV respectively. Here, Patients in Grade IV were showed the habit of maximum frequency of chews per day. [TABLE 4]

The duration of habits varied between 2 – 20 years in OSF patients with mean duration being 3 ± 0.81 years in Grade 1, 3.38 ± 1.06 years in Grade II, 4.38 ± 2.06 years in Grade III and 8.57 ± 3.69 years in Grade IV respectively. Patients in Grade IV were found to be with maximum duration of habit. [TABLE 5]

MOUTH OPENING:

The average mean mouth opening in control group was 51.14 ± 2.54 mm (range 55-48). The mean pre treatment mouth opening in OSF patients was 25.83 ± 10.76 mm (range 10-45 mm) with 40.29 ± 3.15 mm (range 36 – 45 mm) in Grade I, 30.37 ± 2.77 mm (range 26-35 mm) in Grade II, 20.62 ± 3.29 mm (range 16 – 25 mm) in Grade III and 12.14 ± 1.57 mm (range 10-14 mm) in Grade IV respectively. On comparision among control and different grades of OSF patients, these values were found to be statistically significant ($p<0.001$). [TABLE 6]

The post treatment average mean mouth opening in OSF patients was 29.63 ± 10.84 mm (range 13-48 mm) with 43.71 ± 2.87mm (range 41-48 mm) in Grade I, 34.50 ± 3.16 mm (range 30-38 mm) in Grade II, 24.62 ± 4.17 mm (range 19-30 mm) in Grade III, 15.71 ± 2.36 mm (range 13-19 mm) in Grade IV respectively. On comparision among different grades of OSF patients, these values were found to be statistically significant ($p<0.001$). [TABLE 7]

In the present study, on comparison of mean values between the pre treatment and post treatment mouth opening in Grade I, Grade II, Grade III, Grade IV OSF showed highly significant difference in mouth opening ($p<0.001$). The mean difference in mouth opening was 3.8 ± 0.99 mm. The maximum increase in mouth opening was 6 mm and the minimum was 2 mm respectively. [TABLE 8]

BURNING SENSATION:

The control group (n =7) had showed no pain/burning score (100%). The percentage distribution of pain/burning VAS score before the treatment procedure in 30 OSF patients were 2 (5.4 %) had mild pain/burning sensation, 12 (32.4 %) had moderate pain/burning sensation and 16 (43.5 %) had severe pain/burning sensation. [TABLE 9]

The percentage distribution of pain/burning VAS score after the treatment procedure in 30 OSF patients were 26 (86.7 %) had no pain/burning sensation, 4 (13.3 %) had mild pain/burning sensation, 0 (0.0 %) had moderate and severe pain/burning sensation. They showed statistically significant reduction ($p<0.001$) in burning sensation. [TABLE 10]

In the present study, all patients (100 %) in Grade I and Grade II and 7 patients (87.5 %) in Grade III and 4 patients (57.1 %) showed complete reduction in burning sensation after medication whereas 1 (12.5%) patient in Grade III and 3 (42.9 %) in Grade IV showed mild persistance of burning sensation after medication. [TABLE 11]

SERUM HOMOCYSTEINE:

The mean serum homocysteine level in male and female are 19.82 µmol/L and 8.86 µmol/L. The mean serum homocysteine in males was found to be higher than the females. [TABLE 12]

On comparision of mean pre treatment serum homocysteine in males and females, statistically significant difference was noted in Grade I, III and Grade IV patients. There was no female patient in Grade III. No statistically significant difference noted between males and females in control group. On comparision of mean post treatment serum homocysteine in males and females, no statistically significant difference noted in Grsde I, Grade III and Grade IV. [TABLE13].

On comparision between pre and post treatment serum Homocysteine level among males patients, statistically significant difference was noted. No statistically significant difference among female patients in pre and post treatment Homocysteine levels noted [TABLE 14]

The mean serum homocysteine level in control group was 8.40 ±1.74 µmol/L. The mean pre treatment serum homocysteine level in OSF group was 20.67±11.26 µmol/L. The mean serum homocysteine level in Grade I, Grade II, Grade III and Grade IV are 10.69 ± 2.26 µmol/L, 16.94 ± 2.45 µmol/L, 20.44 ± 4.82 µmol/L and 35.17 ± 13.90 µmol/L respectively. [TABLE 15]

On multiple comparison of serum homocysteine between control and 4 grades of OSF. There was no statistically significant difference in serum homocysteine level between control and Grade I and Grade II whereas statistically significant difference in serum homocysteine level between control and Grade IV ($p<0.001$) and Grade III ($p = 0.01$) was found. There was no statistically significant difference in serum homocysteine level between Grade I and Grade II whereas statistically significant difference in serum homocysteine level between Grade I and Grade IV ($p<0.001$) and Grade III ($p = 0.01$) was found. There was no statistically significant difference in serum homocysteine level between Grade II and Grade III whereas statistically significant difference in serum

homocysteine level between Grade II and Grade IV ($p<0.001$) was found. There was statistically significant difference in serum homocysteine level between Grade III and Grade IV ($p =0.001$). [TABLE 16]

Thus the pre treatment mean serum homocysteine levels between control and OSF group showed statistically significant difference ($p<0.001$). The mean serum homocysteine concentration was higher in OSF group when compared to control group. Among the control group and various grades of OSF, the mean serum homocysteine level in Grade IV showed the highest value. Thus with progression of disease from Grade I to Grade IV, a statistically significant increase in mean serum homocysteine level was observed.

The post treatment mean serum homocysteine levels in OSF patients was 14.76 ± 6.45 µmol/L. The mean post treatment serum homocysteine level in Grade I, Grade II, Grade III and Grade IV are 8.18 ± 1.44 µmol/L, 13.98 ±3.32 µmol/L, 15.35 ± 3.70 µmol/L and 21.57 ± 8.02 µmol/L respectively. On comparison, the post treatment mean serum homocysteine levels between different grades of OSF group showed statistically significant difference ($p<0.001$). [TABLE 17]

On multiple comparison of post treatment serum homocysteine between 4 grades of OSF, there was no statistically significant difference in post treatment serum homocysteine level between Grade I Grade II and Grade III whereas statistically significant difference in serum homocysteine level between Grade I and Grade IV ($p<0.001$) was found. There was no statistically significant difference in post treatment serum homocysteine level between Grade II and Grade III whereas statistically significant difference between Grade II and Grade IV ($p = 0.02$) was found. There was no

statistically significant difference in post treatment serum homocysteine level between Grade III and Grade IV. [TABLE 18]

In the present study, comparision of mean pre treatment and post treatment serum homocysteine in OSF patients showed statistically significant difference ($p<0.001$). On comparison among the grades of OSF such Grade I, Grade II, Grade III and Grade IV also showed statistically significant difference ($p=0.009$, $p<0.001$,$p= 0.002$ and $p=0.002$) respectively. [TABLE 19, 20]

The mean difference in serum homocysteine level in Grade I, Grade II, Grade III and Grade IV patients after treatment are 2.50, 2.95, 5.08 and 13.60 respectively.

There was significant reduction in mean serum homocysteine level in OSF patient after treatment was noted ($p=0.003$). The greatest reduction in serum homocysteine was noted in Grade IV patients after medical intervention. [TABLE 21]

Serum homocysteine was compared with variables such as chewing frequency, chewing duration and mouth opening using Pearson correlation. No statistically significant difference were noted when the serum homocysteine levels were correlated with the chewing frequency of habits. Statistically significant difference were noted when the serum homocysteine levels were correlated with chewing duration of habits ($p<0.001$).Statistically significant difference were noted when the serum homocysteine levels were correlated with pretreatment mouth opening ($p<0.001$). Comparison of improvement in mouth opening and serum homocysteine level after treatment was evaluated in all grades of OSF and found highly significant result ($p<0.001$). The serum homocysteine levels were found to be lowering with improvement in mouth opening. [TABLE 22]

TABLE 1: AGE DISTRIBUTION AMONG CONTROL AND VARIOUS GRADES OF OSF

Age (years)	Control	Grade I	Grade II	Grade III	Grade IV	Total
20-30	2	2	4	5	4	17
30-40	3	4	4	2	2	15
40-50	2	1	0	1	1	5
Total	7	7	8	8	7	37

TABLE 2: COMPARISON OF MEAN AGE BETWEEN CONTROL AND VARIOUS GRADES OF OSF

Group	N	Mean	Std. Dev	F-Value	P-Value
Healthy	7	34.86	7.862	0.615	0.655
Grade I	7	32.71	5.122		
Grade II	8	28.88	7.624		
Grade III	8	31.13	7.511		
Grade IV	7	30.86	9.957		
Total	37	31.59	7.588		

TABLE 3: GENDER DISTRIBUTION AMONG CONTROL AND VARIOUS GRADES OF OSF

Gender	GRADE					
	Healthy	Grade I	Grade II	Grade III	Grade IV	Total
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Male	6(85.7)	5(71.4)	8(100)	7(87.5)	6(85.7)	32(86.5)
Female	1(14.3)	2(28.6)	0(0)	1(12.5)	1(14.3)	5(13.5)
Total	7(100)	7(100)	8(100)	8(100)	7(100)	37(100)

TABLE 4: COMPARISON OF MEAN CHEWING FREQUENCY BETWEEVARIOUS GRADES OF OSF

Variable	Grade	Mean	Std. Dev	F-Value	P-Value
Chewing Frequency	Grade I	5.14	2.795	1.632	0.206
	Grade II	6.50	4.309		
	Grade III	8.13	3.643		
	Grade IV	9.71	5.407		
	Total	7.37	4.271		

TABLE 5: COMPARISON OF MEAN CHEWING DURATION BETWEEN VARIOUS GRADES OF OSF

Variable	Grade	Mean	Std. Dev	F-Value	P-Value
Chewing Duration	Grade I	3.00	.816	9.821	***<0.001***
	Grade II	3.38	1.061		
	Grade III	4.38	2.066		
	Grade IV	8.57	3.690		
	Total	4.77	3.014		

TABLE 6: COMPARISON OF MEAN PRE TREATMENT MOUTH OPENING BETWEEN CONTROL GROUP AND VARIOUS GRADES OF OSF

Variable	Group	Mean	Std. Dev	F-Value	P-Value
Pre treatment Mouth Opening	Healthy	51.14	2.54	223.697	**<0.001**
	Grade I	40.29	3.15		
	Grade II	30.37	2.77		
	Grade III	20.62	3.29		
	Grade IV	12.14	1.57		
	Total	30.62	13.98		

TABLE 7: COMPARISON OF MEAN POST TREATMENT MOUTH OPENING BETWEEN VARIOUS GRADES OF OSF

Variable	Group	Mean	Std. Dev	F-Value	P-Value
Post treatment Mouth Opening	Grade I	43.71	2.87	98.872	***<0.001***
	Grade II	34.50	3.16		
	Grade III	24.62	4.17		
	Grade IV	15.71	2.36		
	Total	29.63	10.84		

TABLE 8: COMPARISON OF MEAN PRE AND POST TREATMENT MOUTH OPENING BETWEEN VARIOUS GRADES OF OSF

	n	Mean	Std. Dev	t-Value	P-Value
Mouth opening: Pre	30	25.83	10.76	20.886	***<0.001***
Mouth opening: Post	30	29.63	10.84		

TABLE 9: COMPARISON OF PRE TREATMENT BURNING SENSATION BETWEEN CONTROL GROUP AND VARIOUS GRADES OF OSF

Pre treatment Burning Sensation	**Grade**					
	Healthy	**Grade I**	**Grade II**	**Grade III**	**Grade IV**	**Total**
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
No pain	7(100)	0(0)	0(0)	0(0)	0(0)	7(18.9)
Mild	0(0)	1(14.3)	1(12.5)	0(0)	0(0)	2(5.4)
Moderate	0(0)	3(42.9)	5(62.5)	4(50)	0(0)	12(32.4)
Severe	0(0)	3(42.9)	2(25)	4(50)	7(100)	16(43.2)
Total	7(100)	7(100)	8(100)	8(100)	7(100)	37(100)

TABLE 10: COMPARISON OF POST TREATMENT BURNING SENSATION BETWEEN VARIOUS GRADES OF OSF

Post treatment burning sensation	Grade											
	Healthy		Grade I		Grade II		Grade III		Grade IV		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
No pain	0	0	7	100	8	100	7	87.5	4	57.1	26	86.7
Mild	0	0	0	0	0	0	1	12.5	3	42.9	4	13.3
Moderate	0	0	0	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	7	100	8	100	8	100	7	100	30	100

TABLE 11: PROPORTION CHANGE IN PRE AND POST TREATMENT BURNING SENSATION

Pre treatment Burning sensation	Post treatment Burning sensation					
	No pain		Mild		Total	
	n	%	n	%	n	%
Mild	2	100	0	0	2	100
Moderate	12	100	0	0	12	100
Severe	12	75	4	25	16	100
Total	26	86.7	4	13.3	30	100

TABLE 12: COMPARISON OF MEAN PRE TREATMENT SERUM HOMOCYSTEINE BETWEEN MALES AND FEMALES

	Gender	n	Mean	Std. Dev	t-Value	P-Value
Homocysteine	Male	32	19.8281	11.36486	2.126	0.041
	Female	5	8.8600	2.43475		

TABLE 13: GENDERWISE COMPARISON OF MEAN PRE AND POST TREATMENT SERUM HOMOCYSTEINE

Grade	Variables	Gender	n	Mean	Std. Dev	t-Value	P-Value
Healthy	Homocysteine: Pre	Male	6	8.7833	1.54844	1.604	0.170
		Female	1	6.1000	.		
Grade I	Homocysteine: Pre	Male	5	11.8800	1.13004	4.717	**0.005**
		Female	2	7.7000	.70711		
	Homocysteine: Post	Male	5	8.6400	1.41527	1.456	0.205
		Female	2	7.0250	.88388		
Grade II	Homocysteine: Pre	Male	8	16.9375	2.45004	-	-
		Female	0	.	.		
	Homocysteine: Post	Male	8	13.9813	3.32501	-	-
		Female	0	.	.		
Grade III	Homocysteine: Pre	Male	7	21.7286	3.39299	2.847	0.029
		Female	1	11.4000	.		
	Homocysteine: Post	Male	7	16.1714	3.10576	1.979	0.095
		Female	1	9.6000	.		
Grade IV	Homocysteine: Pre	Male	6	39.1333	9.99713	2.568	0.050
		Female	1	11.4000	.		
	Homocysteine: Post	Male	6	23.8333	5.83872	2.512	0.054
		Female	1	7.9900	.		

TABLE 14: COMPARISON OF PRE AND POST TREATMENT SERUM HOMOCYSTEINE AMONG MALES AND FEMALES

Gender	Variable	N	Mean	Std. Dev	t-Value	P-Value
Male	Homocysteine: Pre	26	22.3769	11.11962	5.665	***<0.001***
	Homocysteine: Post	26	15.8173	6.27594		
Female	Homocysteine: Pre	4	9.5500	2.17486	2.528	0.086
	Homocysteine: Post	4	7.9100	1.31785		

TABLE 15: COMPARISON OF MEAN PRE TREATMENT SERUM HOMOCYSTEINE BETWEEN CONTOL AND VARIOUS GRADES OF OSF

Variable	Group	Mean	Std. Dev	F-Value	P-Value
Pre treatment Serum Homocysteine	Healthy	8.40	1.74	17.765	***<0.001***
	Grade I	10.69	2.26		
	Grade II	16.94	2.45		
	Grade III	20.44	4.82		
	Grade IV	35.17	13.90		
	Total	18.34	11.24		

TABLE 16: MULTIPLE COMPARISON OF MEAN PRE TREATMENT SERUM HOMOCYSTEINE BETWEEN CONTOL AND GRADES OF OSF

Variable	Grade		Mean Difference	P-Value
Pre treatment Serum Homocysteine	Healthy	Grade I	-2.28571	0.967
		Grade II	-8.53750	0.120
		Grade III	-12.03750	0.011
		Grade IV	-26.77143	***<0.001***
	Grade I	Grade II	-6.25179	0.381
		Grade III	-9.75179	0.056
		Grade IV	-24.48571	***<0.001***
	Grade II	Grade III	-3.50000	0.828
		Grade IV	-18.23393	***<0.001***
	Grade III	Grade IV	-14.73393	***<0.001***

TABLE 17: COMPARISON OF POST TREATMENT SERUM HOMOCYSTEINE BETWEEN CONTOL AND VARIOUS GRADES OF OSF

Variable	Group	Mean	Std. Dev	F-Value	P-Value
Post treatment Serum Homocysteine	Grade I	8.18	1.44	9.642	***<0.001***
	Grade II	13.98	3.32		
	Grade III	15.35	3.70		
	Grade IV	21.57	8.02		
	Total	14.76	6.45		

TABLE 18: MULTIPLE COMPARISON OF POST TREATMENT SERUM HOMOCYSTEINE AMONG VARIOUS GRADES OF OSF

Variable	Grade		Mean Difference	P-Value
Post treatment Serum Homocysteine	Grade I	Grade II	-5.80268	0.104
		Grade III	-7.17143	0.031
		Grade IV	-13.39143	**<0.001**
	Grade II	Grade III	-1.36875	0.936
		Grade IV	-7.58875	0.021
	Grade III	Grade IV	-6.22000	0.073

TABLE 19: COMPARISON OF MEAN PRE AND POST TREATMENT SERUM HOMOCYSTEINE IN OSF GROUP

Variable	n	Mean	Std. Dev	t-Value	P-Value
Homocysteine: Pre	30	20.67	11.26	5.618	**<0.001**
Homocysteine: Post	30	14.76	6.45		

TABLE 20: COMPARISONS OF PRE AND POST TREATMENT SERUM HOMOCYSTEINE AMONG VARIOUS GRADES OF OSF

Grade	Variable	n	Mean	Std. Dev	t-Value	P-Value
Grade I	Homocysteine: Pre	7	10.6857	2.25716	3.755	0.009
	Homocysteine: Post	7	8.1786	1.44449		
Grade II	Homocysteine: Pre	8	16.9375	2.45004	6.314	**<0.001**
	Homocysteine: Post	8	13.9813	3.32501		
Grade III	Homocysteine: Pre	8	20.4375	4.81692	4.713	**0.002**
	Homocysteine: Post	8	15.3500	3.69672		
Grade IV	Homocysteine: Pre	7	35.1714	13.89829	5.141	**0.002**
	Homocysteine: Post	7	21.5700	8.01671		

TABLE 21: COMPARISION OF MEAN DIFFERENCE BETWEEN PRETREATMENT AND POSTTREATMENT SERUM HOMOCYSTEINE BETWEEN DIFFERENT GRADES OF OSF

Variable	Group	Mean	Std. Dev	P-Value
Pre and Post treatment serum homocysteine	Grade I	2.5071	1.76645	**0.003**
	Grade II	2.9562	1.32434	
	Grade III	5.0875	3.05307	
	Grade IV	13.6014	6.99972	
	Total	5.9037	5.75534	

TABLE 22: CORELATION BETWEEN PREAND POST TREATMENT HOMOCYSTEINE AND VARIABLES

		Homocysteine: Pre	**Homocysteine: Post**
Chewing Frequency	Correlation	.301	-
	P-Value	.106	
	N	30	
Chewing Duration	Correlation	.903	-
	P-Value	**<0.001**	
	N	30	
Mouth opening: Pre	Correlation	-.781	-.723
	P-Value	**<0.001**	**<0.001**
	N	37	30
Mouth opening: Post	Correlation	-.764	-.737
	P-Value	**<0.001**	**<0.001**
	N	30	30

CHART 1: AGE DISTRIBUTION AMONG CONTROL AND VARIOUS GRADES OF OSF

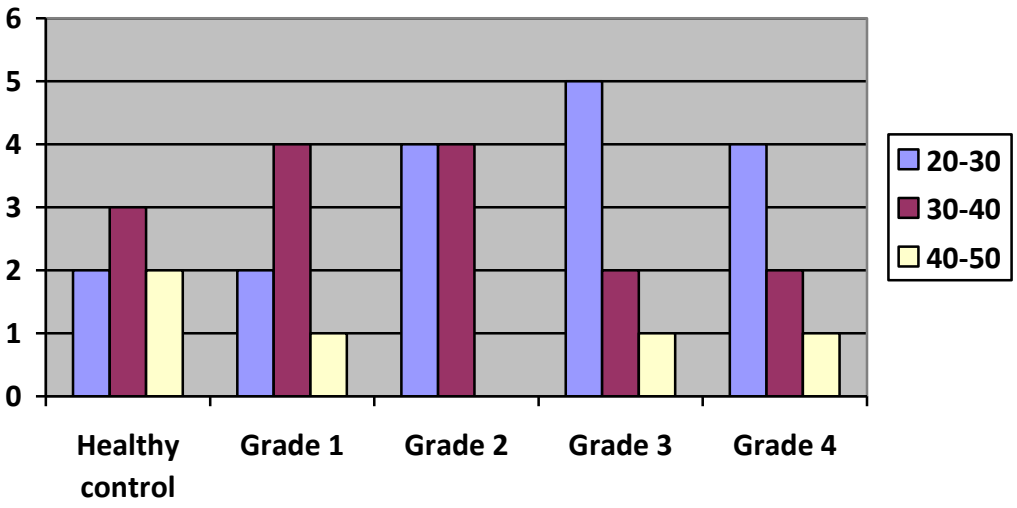


CHART 2: GENDER DISTRIBUTION AMONG CONTROL AND VARIOUS GRADES OF OSF

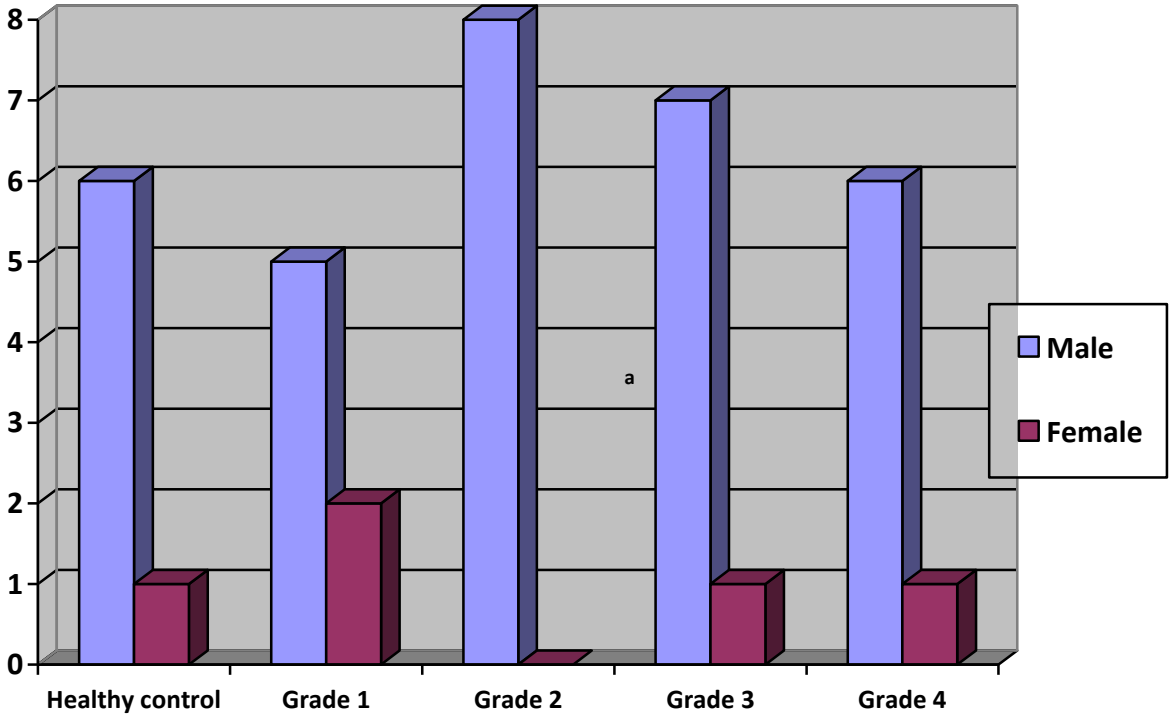


CHART 3: COMPARISON OF MEAN CHEWING FREQUENCY BETWEEN VARIOUS GRADES OF OSF

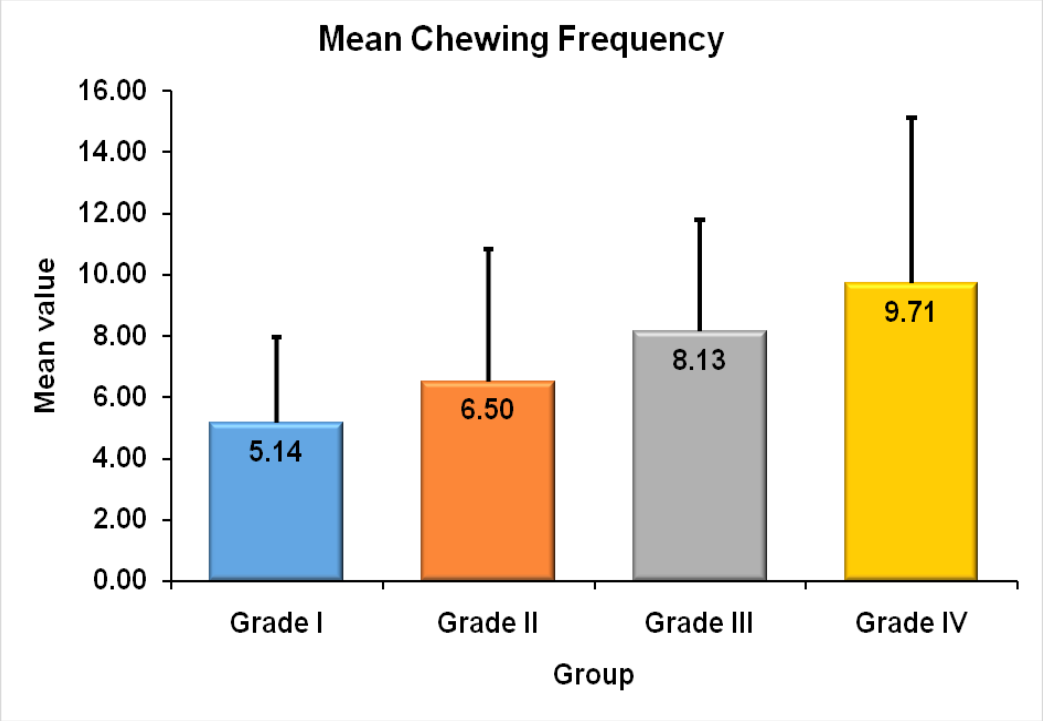


CHART 4: COMPARISON OF MEAN CHEWING DURATION BETWEEN VARIOUS GRADES OF OSF

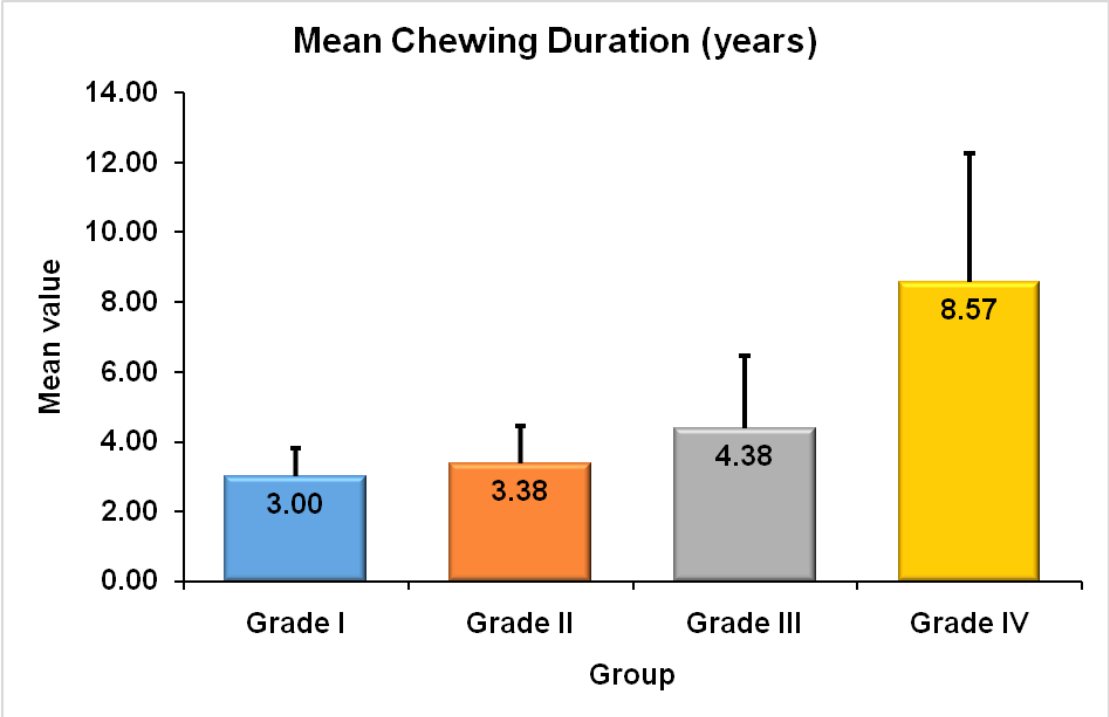


CHART 5: COMPARISON OF MEAN PRE TREATMENT MOUTH OPENING BETWEEN CONTROL GROUP AND VARIOUS GRADES OF OSF

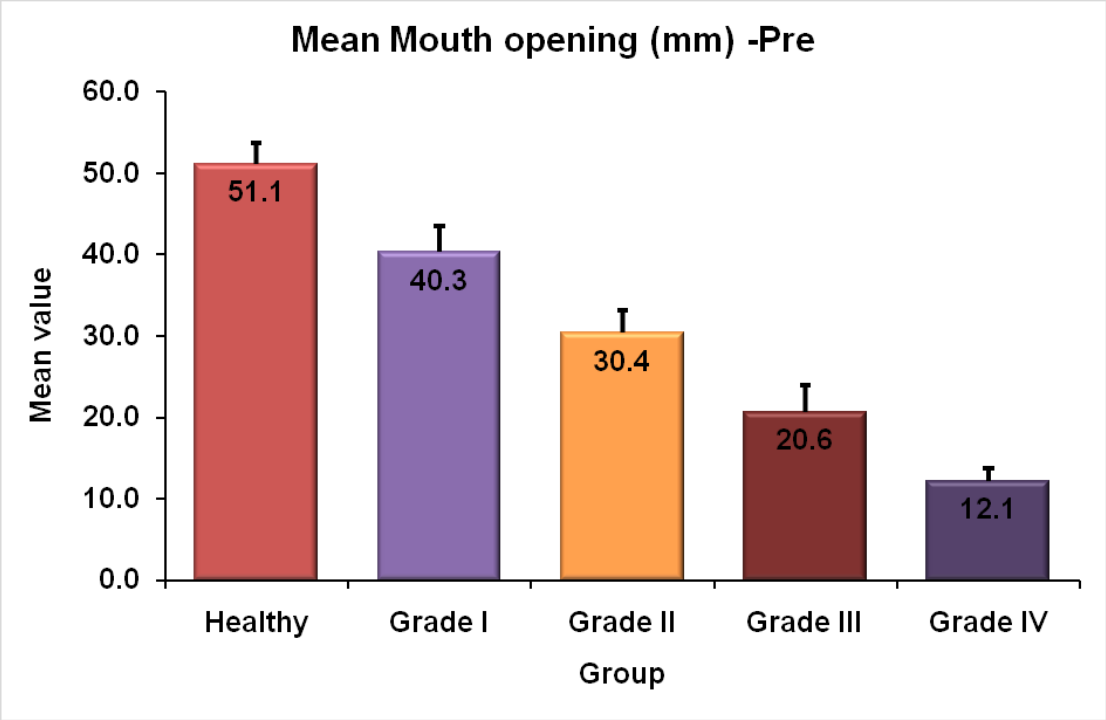


CHART: 6 COMPARISON OF MEAN POST TREATMENT MOUTH OPENING BETWEEN VARIOUS GRADES OF OSF

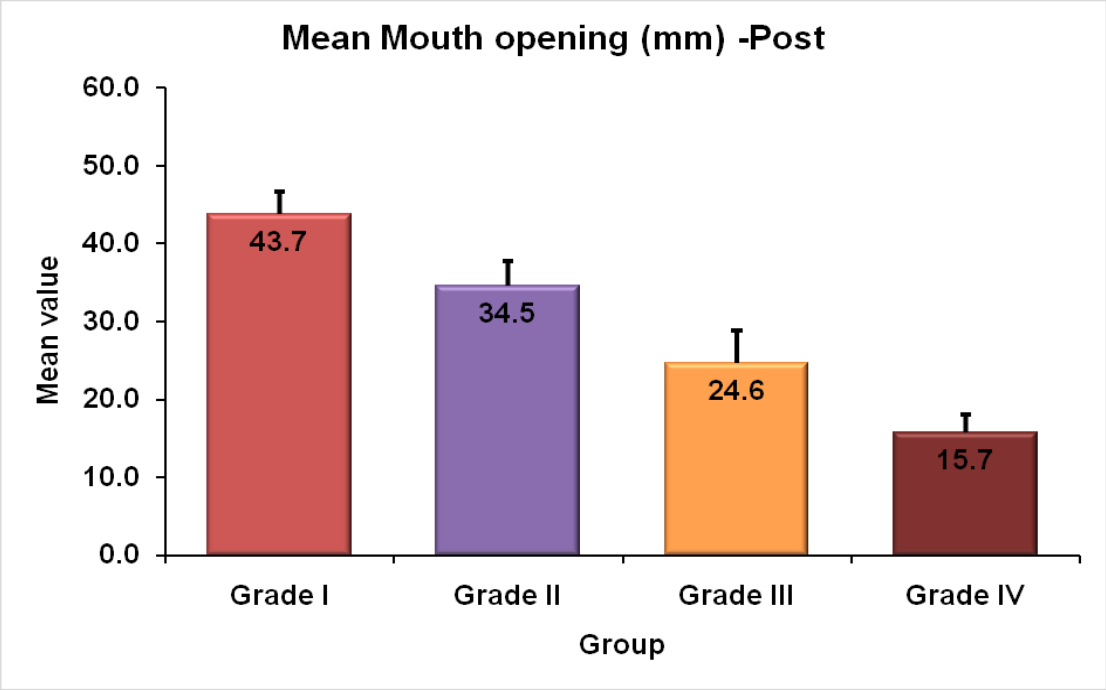


CHART 7: COMPARISON OF PRE TREATMENT BURNING SENSATION BETWEEN VARIOUS GRADES OF OSF

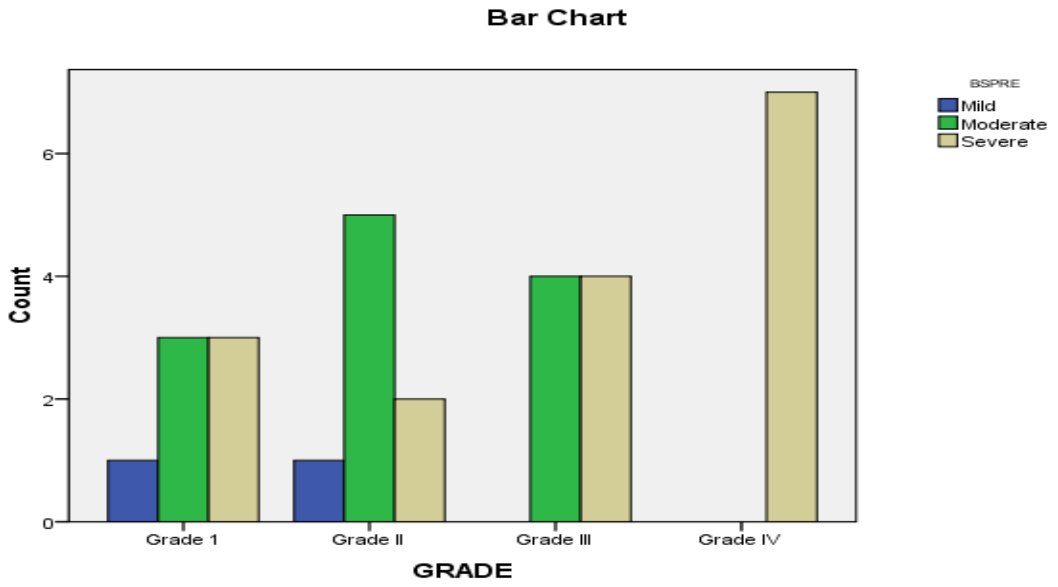


CHART 8: COMPARISON OF POST TREATMENT BURNING SENSATION BETWEEN VARIOUS GRADES OF OSF

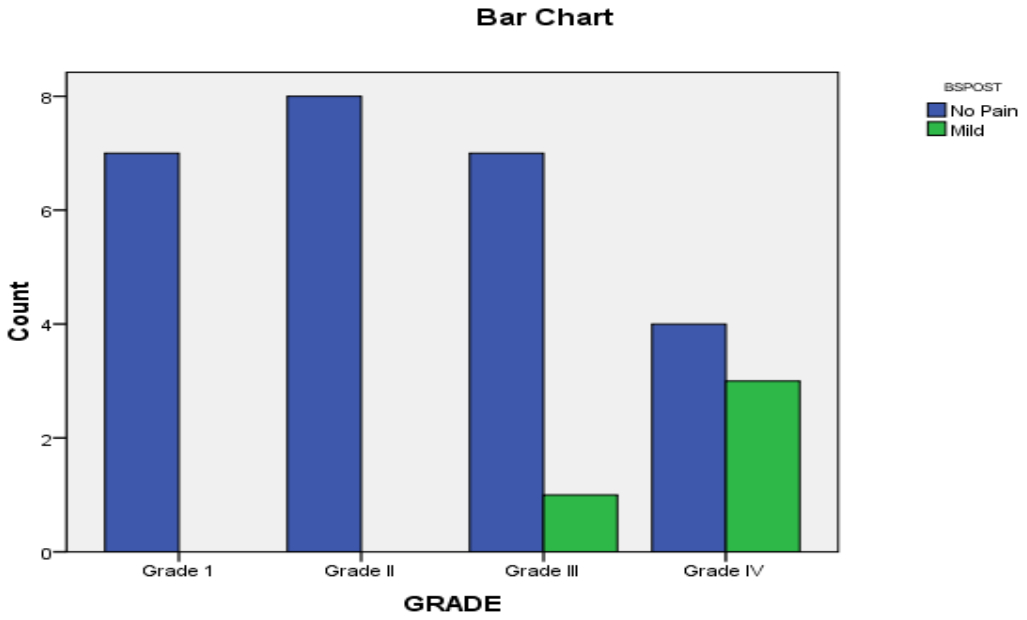


CHART 9: MULTIPLE COMPARISON OF MEAN PRE TREATMENT SERUM HOMOCYSTEINE BETWEEN CONTOL AND GRADES OF OSF

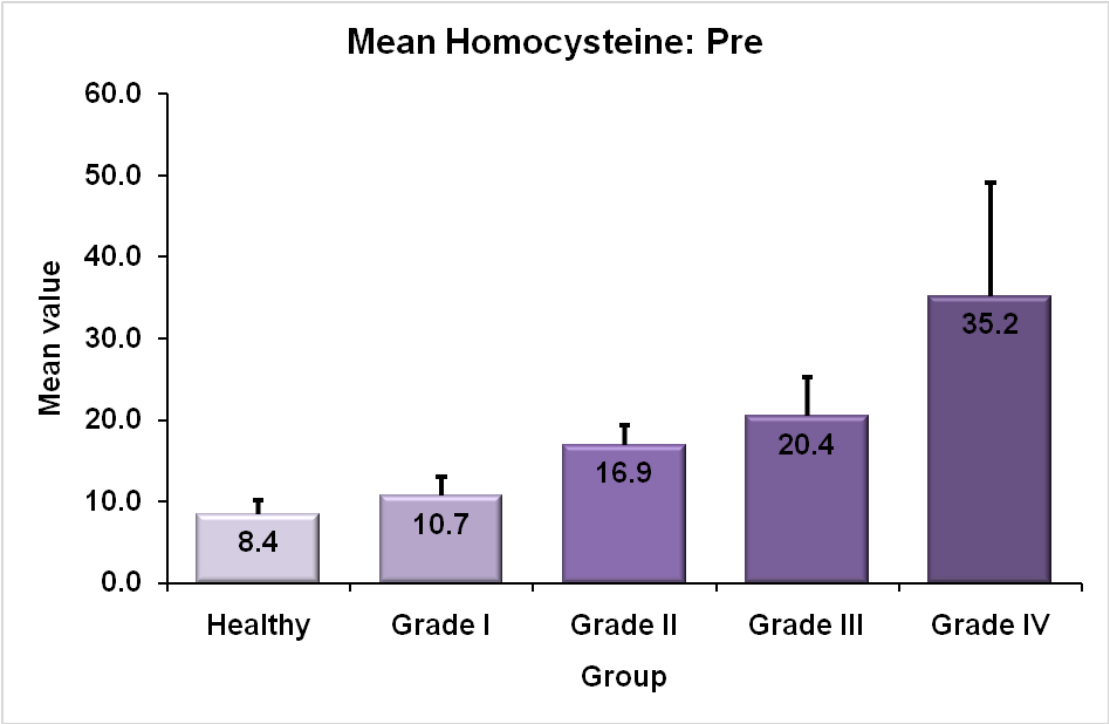


CHART 10: COMPARISON OF POST TREATMENT SERUM HOMOCYSTEINE BETWEEN CONTOL AND VARIOUS GRADES OF OSF

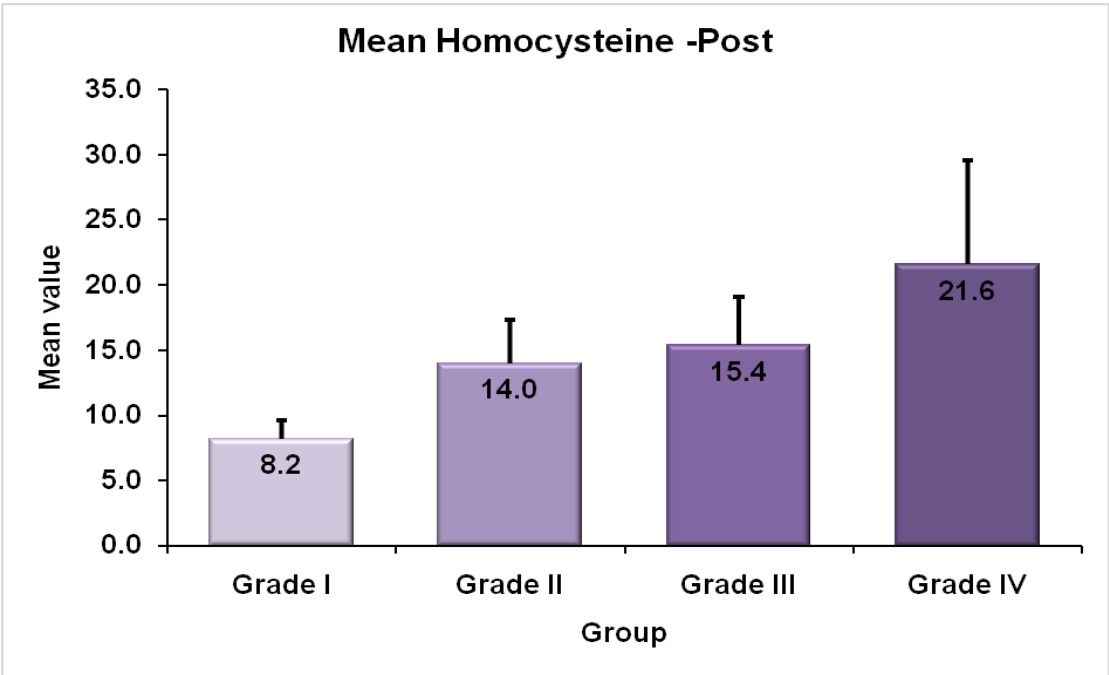


CHART 11: CORELATION BETWEEN PRE TREATMENT HOMOCYSTEINE AND MOUTH OPENING

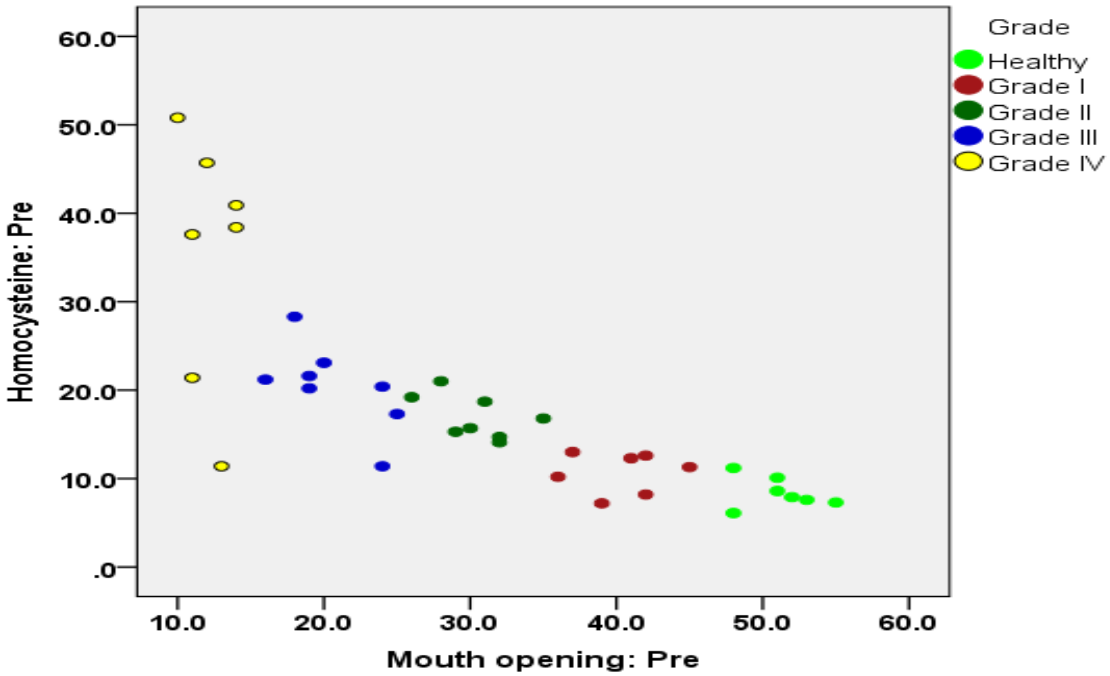
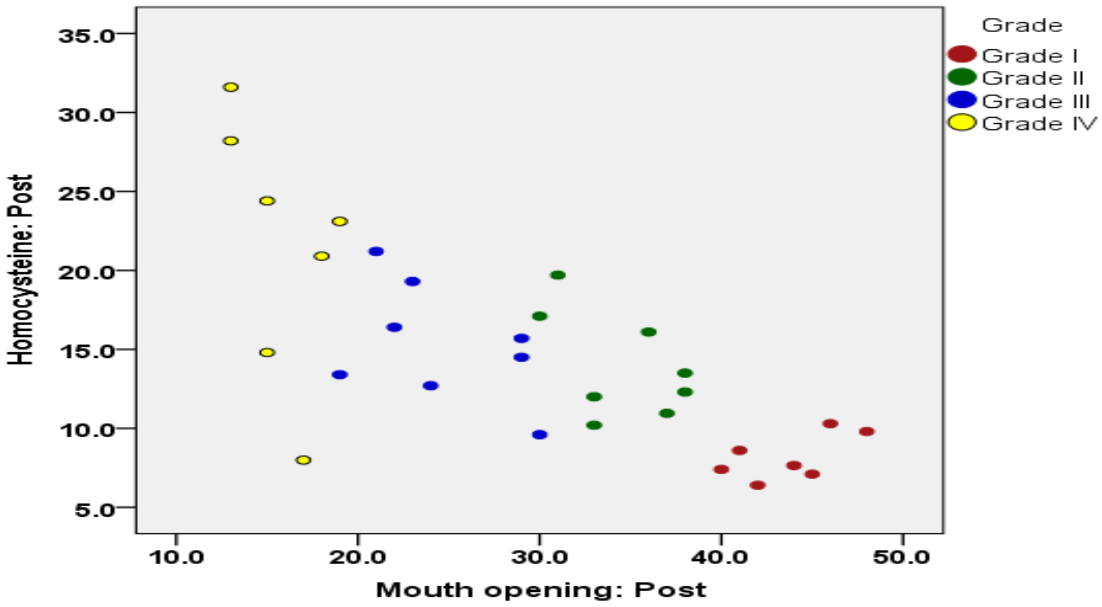


CHART 12: CORELATION BETWEEN POST TREATMENT HOMOCYSTEINE AND MOUTH OPENING



DISCUSSION

Oral submucous fibrosis is a chronic debilitating pre-malignant condition affecting millions of individuals worldwide and is most commonly seen in the Asian subcontinent with increased prevalence in southern parts of the subcontinent[16]. In recent years, the prevalence has increased to 6.42 % in India[17]. OSF is classified under oral potentially malignant disorders[23]. Despite extensive research, the etiology of OSF still remains largely unknown and the pathogenesis is yet to be completely elucidated. Based on clinical, epidemiological and in vitro studies, areca nut chewing is considered as the important predisposing factor[20].

The diffusion of ingredients of arecanut can act as physical irritant to oral mucosa leading to injury related chronic inflammation, oxidative stress and cytokine production[56] and induce juxtaepithelial inflammatory cell infiltration[22] . The excessive production of free radicals and reactive oxygen species is strongly known to activate NF-kappa B, JNK and p38[23]. Moreover vitamin deficiency and malnutrition can derange the repair of the inflamed oral mucosa, leading to defective healing[24].

Homocysteine is sulfur containing non-protein amino acid, derived from methionine, an essential amino acid[1]. Amino acids and their derivatives can reflect the metabolic derangements that occur during the pathological processes induced by the PMDs and cancer[26].

The mechanism of action by which homocysteine causes the disease is complex and not completely understood. Hyperhomocysteinemia is known to exert its detrimental effects through oxidative damage by formation of reactive oxygen species (ROS) and protein homocysteinylation[9]. The oxidative stress is known to induce acute

and chronic inflammation via the regulation of NF-κB transcription factor. Thus hyperhomocysteinemia indicates the presence of inflammation[10].

Inflammation is recognized to play a critical role in the pathogenesis and progression of all life-threatening diseases and varying degrees of hyperhomocysteinemia is detectable in all inflammatory diseases including cardiovascular disease, stroke, renal failure and cancer[10].

OSF is chronic mucosal inflammatory disease; hence control of inflammation or the factors influencing the inflammatory process form the basis of definitive management. The injection of corticosteroids was the most frequently studied medical therapy and in many centers it remains the first line of treatment for symptomatic patients with OSF[39]. Numerous studies have proven that management of premalignant lesions should include along with cessation of habit. Micronutrient supplementation has also proved to be efficacious in the management of oral submucous fibrosis[76]. Since no single drug has provided complete relief of symptoms in the present study combination of drugs used to treat the condition.

Biochemical investigations have been the simple and earliest form of interventions to localize parameters that predispose to the development of the condition, monitor the response to therapy and prognosticate on its progression and malignant transformation potential[25].

Only few studies have been recorded in the literature to show an association of serum homocysteine in oral mucosal diseases, pre malignancies like lichen planus, laryngeal leukoplakia and cervical dysplasia and malignancies like carcinomas of breast, lung and head and neck. To best of our knowledge, the current study is only a fourth of its kind, to evaluate the serum homocysteine levels in OSF and is the first to compare and

analyze the serum homocysteine levels before and after medical intervention. Thus, this study aimed to assess the homocysteine levels in various grades of progression of OSF, to compare the levels between the different groups before and after medical intervention. Hence, this study intended to evaluate whether homocysteine can be used as a therapeutic prognostic marker in oral submucous fibrosis patients.

A total of 37 study participants comprising of 30 OSF patients and 7 healthy controls were included in the study. OSF patients were graded clinically into 4 grades. The mean age of study population was 31.59 ± 7.588. The maximum number of OSF was in the age group of 20 – 40 years. Out of 37 subjects, 32 were males and 5 were females. Among the OSF patients, a male predilection was observed. This is similar to study by **Hazarey et al[17].**

Areca nut is the fourth most addictive substance in the world. The habit of betel quid chewing is widespread throughout India and South East Asia[21] and it is widely prevalent in teenagers and young adults[19.] All the OSF patients in the study had a habit of arecanut chewing in the form of commercial preparation such as mawa or pan masala. Majority of them used mawa which is similar to study by **Sinor et al[48]**.

In OSF patients, the Chewing frequency varied between 2 and 15 per day with mean frequency of 5.14 ± 2.795 per day. Patients in Grade IV showed the maximum frequency of chews per day. The duration of habit varied between 2 – 20 years with mean duration being 3 ± 0.81 years. Patients in Grade IV were found to be with maximum duration of habit. The habit variables showed significant correlation to severity to clinical grading which is similar to study by **Reddy et al[104]**.

The most important measures of outcome associated with morbidity OSF is pain/burning sensation to spicy foods, inability to open the mouth with progression of

fibrosis[39].In the present study, the control group showed no pain/burning score. The percentage distribution of pain/burning VAS score before the treatment procedure in 30 OSF patients were 5.4 % had mild pain/burning sensation, 32.4 % had moderate pain/burning sensation and 43.5 % had severe pain/burning sensation.

After medical intervention, the proportion change between pre and post treatment burning sensation was found to be 86.7 % with no pain and 13.3 % showed mild persistance of pain. 100% patients in Grade I and Grade II and 87.5 % patients in Grade III and 57.1 % patients in Grade IV showed complete reduction in burning sensation whereas12.5% patient in Grade III and 42.9 % in Grade IV showed mild persistance of burning sensation. Studies by **Gupta et al** showed similar results of 82%, 82% and 51% reduction of burning sensation with biweekly injection of intralesional steroids.

The average mean mouth opening in control group was 51.14 ± 2.54 mm and OSF patients was 25.83 ± 10.76 mm with severe reduction of mouth opening in Grade IV patients. After medical intervention, average mouth opening in OSF patients was improved to 29.63 ± 10.84 mm. The mean difference in mouth opening was 3.8 ± 0.99 mm. On comparision among different grades of OSF patients, these values were found to be statistically significant ($p<0.001$).

According to **Maher R et al[75]** multiple minerals and micronutrients showed significant improvement in mouth opening and reduction of burning sensation. **Kumar et al[76]** and showed significant improvement in mouth opening and burning sensation either singly with antioxidant lycopene or in association with intralesional steroids. Studies by **Gupta et al** showed reduction of burning sensation and improvement of mouth opening with biweekly injection of intralesional steroids.

In the present study, the mean serum homocysteine of control and OSF patients was 8.40 ± 1.74 and 20.67 ± 11.26. On comparison with mean age, increased serum homocysteine was noted in OSF group compared to control group with statistically significant difference. The reference range of total Hcy for age of 35 years is 10± 3.5µmol/L^2. This indicates hyperhomocysteinemia in OSF group. **Jaganath et al**[26] reported homocysteine levels greater than 20µmol/L in all the OSF patients.

In the present study, no statistically significant difference noted between males and females in control group. The mean pretreatment serum homocysteine levels in male and female patients are 19.82µmol/L and 8.86µmol/L. The serum homocysteine in males was found to be higher than the females. Statistically significant difference was noted in Grade I, III and Grade IV patients. There was no female patient in Grade II. Results were similar to the studies performed by **Bias et al[102], Narang et al[103] and Jaganath et al**[26].

On comparison between the control group and various grades of OSF, the mean serum homocysteine level in Grade IV showed the highest value. Thus with progression of disease from Grade I to Grade IV, a statistically significant increase in serum homocysteine level was observed. This is similar to the study by **Bias et al[102]** and **Narang et al[102]** who reported higher value in Grade IV than Grade III and Grade II but the difference was not found to be statistically significant. **Jaganath et al**[26] reported statistically significant difference between stage I and Stage II with gradual increase from stage II to stage III.

The post treatment mean serum homocysteine levels in OSF patients was 14.76 ± 6.45 µmol/L showed a statistically significant difference ($p<0.001$). The mean difference in serum homocysteine level in Grade I, Grade II, Grade III and Grade IV

patients after treatment are 2.50, 2.95, 5.08 and 13.60 respectively. Thus, greater reduction serum homocysteine level after medical intervention was noted.

Post treatment comparision showed no statistically significant difference in level of Hcy between male and female and clinical grading. On comparision between pre and post treatment serum Homocysteine level among males patients showed statistically significant difference whereas among females patients showed no statistically significant difference. This might be due to very few female patients in sample.

In the present study, serum homocysteine was compared with variables such as chewing frequency, chewing duration and mouth opening using Pearson correlation. No statistically significant difference were noted with the chewing frequency where as chewing duration showed statistically significant difference ($p<0.001$) with longer duration of chewing habit was found to be strongly associated with increased serum homocysteine levels. Studies by **Jaganath et al**[26] showed negative correlation with chewing frequency and chewing duration. **Chao MC etal**[83] reported elevated homocysteine levels in betel nut chewers compared to subjects with normal homocysteine levels.

Serum homocysteine was correlated with the preoperative and postoperative mouth opening which showed with improvement in mouth opening, statistically significant reduction in levels of serum homocysteine.

Glucocorticoids cause a reduction in collagen fibres by inhibiting the proliferation of fibroblast and act to release cellular proteases to activate the collagenase to stimulate the rate of collagen breakdown. They also act by inhibiting the inflammatory response[39]. Regulation and control of NF-kB activation can be a powerful therapeutic strategy for inhibiting tumor growth and for reducing the tissue damage that follows the

release of inflammatory mediators. Studies have shown the immunosuppressive and anti-inflammatory actions of glucocorticosteroids are potent inhibitors of NF-kB activation in mice and cultured cells[105].

The antioxidant vitamins are employed to stabilize and deactivate the free radicals before they attack cells[73]. **Kalikiri PC[106]** in a review discussed that regardless of the cause of hyperhomocysteinemia, most patients derive some benefit from vitamin supplementation via the conversion of homocysteine back to methionine or cysteine. Homocysteine levels usually decrease after a few weeks of therapy and normalize within six to eight week. Several studies have shown that supplementation of dietary folate and Vit B12 was efficient in bringing down Hcy levels.

Ubbink et al[107] in a placebo controlled study showed the combination of vitamin supplementation was very effective in reducing homocysteine levels. **Kim and Pae et al** in an in vitro study showed that the toxic influence of Hcy can be blocked by antioxidative enzyme supplementation. **Racek et al[86]** in a randomized study showed folic acid as more effective Hcy lowering agent and antioxidant did not influence Hcy concentration but improved antioxidative defence. **Sun A et al (2013)[90]** conducted a study in 399 patients with burning mouth syndrome. They were treated with vitamin supplements and found complete remission of oral symptoms with significant reduction in serum homocysteine levels. **Sun A et al (2012)[89]** conducted a study in 91 atrophic glossitis patients to evaluate whether supplementation of different vitamins and iron could reduce the serum homocysteine levels and found significant reduction in homocysteine levels.

SUMMARY

Oral submucous fibrosis is a common pre-malignant condition affecting the oral mucosa with more prevalence among Indian population. Biochemical investigations are the simple and earliest form of interventions to localize parameters that predispose to the development of the condition, monitor the response to therapy and prognosticate on its progression. Homocysteine is a non essential amino acid and altered levels noted in various mucosal disease and head and neck cancers. The study was conducted to evaluate serum homocysteine level as a therapeutic prognostic marker in oral submucous fibrosis.

To the best of our knowledge, the current study is only a fourth of its kind, to evaluate the serum homocysteine levels in OSF and is the first to compare and analyze the serum homocysteine levels before and after medical intervention.

A total of 37 study participants comprising of 30 OSF patients and 7 healthy controls were included in the study. After clinical examination, OSF patients were graded clinically into 4 grades. All the participants in control group and OSF group were subjected to Homocysteine evaluation. OSF patients in Grade I stage were given supplemental medication whereas patients in Grade II, Grade III and Grade IV were administered intralesional injection of steroids twice weekly with supplemental medication for 6 weeks. Patients were evaluated for improvement in signs and symptoms such as burning sensation and mouth opening and were recorded after the complete course of treatment. All the patients were subjected to post operative Homocysteine evaluation and the values are recorded. All the values were statistically analysed and the results were drawn.

The results revealed that the mean homocysteine was higher in OSF group compared to control group. On comparision among different grades of OSF patients,

these values were found to be statistically significant. The mean homocysteine in males was higher than females. The homocysteine level was higher in Grade IV. With progression of disease from Grade I to Grade IV, a statistically significant increase in mean serum homocysteine level was observed. There was significant reduction in mean serum homocysteine level in OSF patient after treatment was noted. On comparision of mean pre treatment and post treatment serum homocysteine showed statistically significant difference.

CONCLUSION

The present study was conducted to evaluate the serum homocysteine level in patients with oral submucous fibrosis in various stages before and after medical intervention and to compare and assess the serum homocysteine level as prognostic marker in Oral Submucous Fibrosis.

In the study, serum homocysteine level was higher in OSF group when compared to control group with statistically significant differences. Longer duration of chewing habit showed increased serum homocysteine levels. Serum homocysteine level in Grade IV showed the highest value. Thus, with progression of disease from Grade I to Grade IV, a statistically significant increase in serum homocysteine level was observed.

After medical intervention, there was statistically significant reduction in serum homocysteine levels in all the grades of OSF. Statistically significant correlation between reduction in serum homocysteine and improvement in mouth opening was noted. However, there was no significant correlation between Homocysteine and burning sensation. On comparison between the pretreatment and post treatment, statistically significant difference in serum homocysteine level was noted in all the grades of OSF.

So, Serum Homocysteine level may be contributory as a potential prognostic marker in treatment of Oral submucous fibrosis.

However, studies with larger sample size are needed to reinforce the findings of our study. We have not included Grade IV B - Advanced stage with premalignant and malignant changes such as Oral submucous fibrosis with Leukoplakia and Squamous cell carcinoma and future studies including Grade IV B may prove more helpful in throwing more light on serum Homocysteine as a prognostic marker in the treatment of Oral submucous fibrosis.

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TAMIL NADU GOVRNMENT DENTAL COLLEGE & HOSPITAL, CHENNAI – 3.

TELEPHONE : 044-253403343
FAX: 044- 25300681
date : 24/09/2015

Ref No: R.C No.0430/DE/2015 dated 27.01.2015, O/O Principal, TNGDC
Sub: IEC review of the research proposals,

Title of the work: Evaluation of serum homocysteine as prognostic marker of oral submucous fibrosis

Principal Investigator: Dr. M. Gayathri
II Yr. M.D.S., Student.

Department : Department of Oral Medicine and Radiology
Tamil Nadu Govt. Dental College & Hospital , Chennai-3

Thank you for submitting your research proposal , which was considered at the Institutional Ethics Committee meeting held on 02-07-2015, at TN Govt. Dental College and the documents related to the study referred above were discussed and the modifications done as suggested and reported to us through your letter dated 23-09-2015 have been reviewed.

The decision of the members of the committee , the secretary and the Chairperson IEC of TN Govt. Dental College is here under:

Approved	Approved and advised to proceed with the study
Approved with suggestions	----------
Revision	----------
Rejected	----------

The principal investigators and their team are advised to adhere the guide lines given below:

1. You should get detailed informed consent from the patients / participants and maintain confidentiality.
2. You should carry out the work without affecting regular work and without extra expenditure to the Institution or the Government.
3. You should inform the IEC, in case of any change of study procedure, site, and investigating guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the institution(s) .
6. You should complete the work within specific period and if any extension of time is required, you should apply for permission again to do the work.
7. You should submit the summary of the work to the ethical committee every 3 months and on completion of the work.
8. You should not claim any kind of funds from the institution for doing the work or on completion/ or for any kind of compensations.
9. The members of the IEC have the right to monitor the work without prior intimation.
10. Your work should be carried out under the direct supervision of the guide/ Professor.

MEMBER SECRETARY,
INSTITUTIONAL ETHICS COMMITTEE
Tamil Nadu Govt. Dental College & Hospital
Chennai

CHAIRPERSON
INSTITUTIONAL ETHICS COMMITTEE
Tamil Nadu Govt. Dental College & Hospital
Chennai

ஆராய்ச்சி பற்றிய தகவல் படிவம்

மரு.ம.காயத்ரி ஆகிய நான் மரு.க.வெ.முரளி கோபிகா மனோகரன், MDS அவர்களின் வழிநடத்துதலின் கீழ் "ஊணீரில் உள்ள ஹோமோசிஸ்டின் அளவின் மதிப்பீடு மூலம் வாய் இறுக்கு நோய் குணமடைதலை முன்கணிப்பு செய்தல் பற்றிய ஆய்வு."

ஆய்வின் நோக்கம்

இந்த ஆய்வின் நோக்கமானது, வாய் இறுக்கு நோயாளிகளின் ஊணீரில் உள்ள ஹோமோசிஸ்டின் அளவை சிகிச்சைக்கு முன் மற்றும் சிகிச்சைக்குப் பின் மதிப்பிட்டு கண்டறிதல்.

செய்முறை

ஆய்வில் பங்கேற்கும் வாய் இறுக்கு நோயாளிகளுக்கு, முழுமையான மருத்துவ வரலாறு அறியப்பட்டு, முழுவாய் பரிசோதனை செய்யப்படும். முந்திய விலாவின் உட்பகுதியில் தமனியிலிருந்து ஊசி மூலம் 3 மி.லி. இரத்தம் எடுக்கப்பட்டு, பின் ஆய்வு கூடத்திற்கு ஊணீரில் உள்ள ஹோமோசிஸ்டின் மதிப்பீட்டிற்கு அனுப்பி வைக்கப்படும். ஆய்வில் பங்கேற்கும் வாய் இறுக்கு நோயாளிகளுக்கு வாரத்திற்கு இருமுறை வாயின் உட்புறத்தில் 2 மி.லி. டெக்ஸாமீதாசோன் மற்றும் 0.5 மி.லி. லிக்னோகெயின் ஊசி போடப்படும். கூடுதலாக ஆண்டி ஆக்ஸிடன்டுகள் அடங்கிய கூட்டு மருந்து கொடுக்கப்படும். ஆறு வாரங்கள் சிகிச்சைக்கு பின் மீண்டும் ஊணிரிலுள்ள ஹோமோசிஸ்டின் மதிப்பீட்டிற்கு இரத்தம் எடுக்கப்பட்டு ஆய்வு கூடத்திற்கு அனுப்பி வைக்கப்படும்.

நன்மைகள்

ஊணுரிலுள்ள ஹோமோசிஸ்டின் அளவைக் கொண்டு வாய் இறுக்கு நோயுள்ளவர்களின் நோய், சிகிச்சைக்குப் பின் குணமடைந்து உள்ளதா என்பதை அறிய உதவியாக இருக்கும்.

இரகசியத் தன்மை

நோயாளிகள் பற்றிய குறிப்புகள் ஆராய்ச்சி முடியும் வரை ரகசியமாக பாதுகாக்கப்படும். இந்த ஆராய்ச்சியை வெளியிடும் போது நோயாளிகளின் தனிப்பட்ட விவரங்கள் எதுவும் பாதிக்கப்படமாட்டாது.

பங்குபெறுவோரின் உரிமை

இந்த ஆராய்சியில் பங்குபெறுவது நோயாளிகளின் தனிப்பட்ட விருப்பம். மேலும், நோயாளிகள் இந்த ஆராய்ச்சிலிருந்து எப்போது வேண்டுமென்றாலும் விலகிக்கொள்ளலாம். நோயாளிகளின் இந்த முடிவினால் அவருக்கோ அல்லது ஆராய்ச்சியாளருக்கோ எவ்வித பாதிப்பும் கிடையாது.

இந்த ஆராய்ச்சியின் முடிவுகள் நோயாளிகளுக்கு ஆராய்ச்சியின் இடையிலோ அல்லது முடிவிலோ தெரிவிக்கப்படும். இதில் ஏதேனும் பின் விளைவுகள் ஏற்பட்டால் அதை சரிசெய்ய சிகிச்சை அளிக்க தகுந்த உதவிகள் செய்யப்படும்.

இழப்பீடு

எதுவும் வழங்கப்படமாட்டாது.

ஆய்வு பற்றிய தகவல் பெற

மரு.ம.காயத்ரி,
இரண்டாம் ஆண்டு, MDS, முதுநிலை மாணவி,
வாய்நோய் அறிதல் மற்றும் ஊடுகதிர் துறை,
தமிழ்நாடு பல்மருத்துவ கல்லூரி மற்றும் மருத்துவமனை,
சென்னை-600 003.
தொலைபேசி : 94448 20486

நோயாளியின் பெயர் **கையொப்பம்/கைரேகை**

ஆராய்ச்சியாளரின் பெயர் கையொப்பம்

தேதி

PATIENT INFORMATION SHEET

I, Dr. M. Gayathri, II year MDS student, Department of Oral Medicine And Radiology, primary investigator under the guidance of Prof. Dr. G.V. Murali Gopika Manoharan, MDS, Professor, Department of Oral Medicine And Radiology, Tamil Nadu Government Dental College and Hospital, have planned to conduct a study titled "Evaluation of Serum Homocysteine as Prognostic Marker of Oral Submucous Fibrosis" in Tamilnadu Government Dental College and Hospital, Chennai – 3

Purpose of the study

We are conducting this study to estimate and compare the serum homocysteine levels in patients with oral submucous fibrosis in various stages before and after treatment.

Procedures

Complete medical history, oral cavity examination and blood investigation will be done.Blood sample is collected by vein puncture above the antecubital fossa and 3ml of blood is withdrawn.It is then sent to laboratory for evaluation of serum homocysteine level.Treatment with intralesional injection of 2ml of dexamethasone and 0.5ml of lignocaine is administered at single site bilaterally on buccal mucosa twice weekly for six weeks and commercially available antioxidant medication will be given.After 6 weeks of treatment, blood sample are again collected and sent to laboratory for evaluation of serum homocysteine.

Benefits of participation

1.Treatment for oral submucous fibrosis will be given.

2.If positive results are obtained from this study, estimation of serum homocysteine will be useful in judging the prognosis

Participant's rights

Taking part in this study is voluntary. Patients are free to decide whether to participate in this study or to withdraw at any time; patients decision will not result in any loss of benefits to which you are otherwise entitled.The results of this special study may be intimated to patient at the end of the study period.

Risk of participation

Participants are selected based on proper inclusion and exclusion criteria and patients who are contraindicated to steroids are excluded.The most common currently used medical treatment of oral submucous fibrosis is intralesional injection of 2ml of dexamethasone with 0.5ml of lignocaine and no complications have been encountered.There is risk of candidiasis,transient decreased immunity and weight gain,acneiform skin eruptions and hyperglycemia which is informed to the patient prior to obtaining their consent.If any adverse effect occurs during the course of treatment,immediate proper treatment will be provided.

Confidentiality

The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Compensation

Nil

Contacts for queries related to the study:

Dr.M.Gayathri,
II year PG student,
Dept of Oral Medicine and Radiology,
Tamilnadu Government Dental College,
Chennai 600003
Phone No 9444820486

Name of the Patient **Signature /Thumb impression**

Name of the investigator **Signature**

Date

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு

''உமிழ்நீரில் உள்ள ஹோமோசிஸ்டின் அளவின் மதிப்பீடு மூலம் வாய் இறுக்கு நோய் குணமடைதலை முன்கணிப்பு செய்தல் பற்றிய ஆய்வு.''

ஆராய்ச்சி நிலையம் : அரசு பல் மருத்துவக் கல்லூரி
சென்னை - 600 003

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் எண் :

பங்கு பெறுவரின் பிறந்த தேதி : _____ / _____ /_____
தேதி மாதம் வருடம்

இந்த ஆய்வு சம்பந்தமாக நான் மேலே கூறப்பட்ட தகவல் படிவத்தை முழுமையாக படித்துப் பார்த்தேன் என்று உறுதி கூறுகிறேன்.

நான் இது தொடர்பான அனைத்து கேள்விகளுக்கும் நிறைவான பதில்கள் பெறப்பட்டேன்.

இந்த ஆய்வின் எனது பங்கு தன்னிச்சையானது என்றும் எந்த நேரத்திலும் இந்த ஆய்வில் இருந்து சட்ட உரிமைகள் பாதிக்கப்படாமல் விலகிக் கொள்ள சம்மதிக்கிறேன்.

மருத்துவ ஆய்வு அதிகாரிகள், எனது சிகிச்சை தொடர்பான பதிவேடுகளை பார்வையிடவும் எந்த நேரத்திலும், ஆய்வில் இருந்து நான் விலகினாலும் பார்வையிட சம்மதிக்கிறேன். எனது அடையாள குறிப்புகள் மூன்றாவது நபருக்கு தெரிவிக்கப்படமாட்டாது என்று புரிந்து கொண்டேன்.

இந்த ஆய்வு அறிக்கைகளை பயன்படுத்தவும், வெளியிடவும், நான் சம்மதிக்கிறேன். ஆய்வாளர் எனது மருத்துவக் குறிப்புகளை வெளியிட தடையாக இருக்கமாட்டேன் என உண்மையாக சம்மதிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் ..... இடம்..... தேதி.....

கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம்..... தேதி.....

ஆய்வாளரின் பெயர் .....

Informed Consent Form

"Evaluation of Serum Homocysteine as Prognostic Marker of Oral Submucous Fibrosis"

Participant ID No:

"I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw from the study at any time without in any way it affecting my further medical care."

Date	Name of the participant	Signature/thumb impression of the participant

[The literate witness selected by the participant must sign the informed consent form. The witness should not have any relationship with the research team; If the participant doesn't want to disclose his / her participation details to others, in view of respecting the wishes of the participant, he / she can be allowed to waive from the witness procedure (This is applicable to literate participant ONLY). This should be documented by the study staff by getting signature from the prospective participant]

"I have witnessed the accurate reading of the consent form to the potential participant and the individual has had opportunity to ask questions. I confirm that the individual has given consent freely"

Date	Name of the witness	Signature of the witness

Date	Name of the interviewer	Signature of the interviewer

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

TAMIL NADU GOVT. DENTAL COLLEGE & HOSPITAL, CHENNAI -3

CASE PROFORMA

EVALUATION OF SERUM HOMOCYSTEINE AS PROGNOSTIC MARKER OF ORAL SUBMUCOUS FIBROSIS

Date: Serial no:

Name: O.P No:

Age/Sex:

Address:

Phone no:

Occupation: Income:

Religion:

Centre: Department of Oral Medicine And Radiology,

Tamil Nadu Govt Dental College & Hospital, Chennai -3

Presenting complaint with duration:

Past medical history:

Past Surgical history:

Past dental history:

Personal history:

A) Diet:

B) Teeth cleansing habits:

- Cleaning aids used:
- Frequency :

C) Smoking habit:

- Material used:
- Frequency :
- Duration of the habit:

D) Chewing habit:

- Material used:
- Frequency :
- Duration of the habit:

E) Other habits (alcohol, snuff):

Marital status:

Menstrual History:

Family history:

CLINICAL EXAMINATION

Extraoral Examination:

Facial asymmetry

Temporomandibular joint

Intraoral examination:

Mouth opening (interincisal distance):

Size and Shape of mouth:

Jaw movements:

- Teeth:

- Gingiva:

- Alveolar mucosa:

- Labial and buccal mucosa:

- Hard palate:

- Soft Palate:

- Pillar of fauces and Tonsils:

- Tongue:

- Floor of the mouth:

- Retromolartrigone:

Provisional diagnosis:

Investigations:

1) Laboratory investigations:

   A) Blood:
      RBC Count:
      Total WBC count:
      Differential count: P L E Bleeding time:
      Haemoglobin %: Clotting time:
      Peripheral smear:
      Erythrocyte sedimentation rate:

   B) Urine:
      Glucose:

Clinical diagnosis:

Treatment plan:

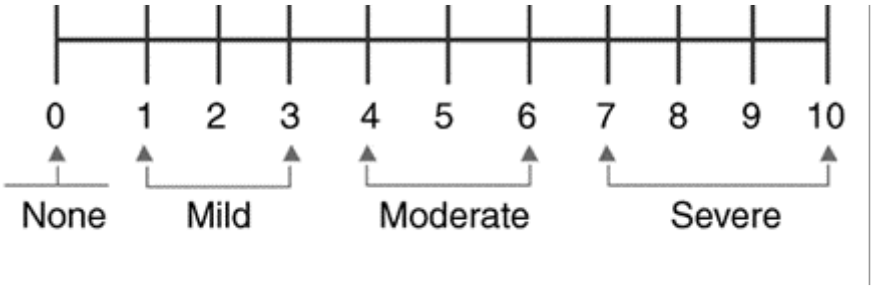
ASSESMENT FORM

Name: **Age/Sex:** **Op.No:** **Serial No:**

Diagnosis: **Grade:**

CLINICAL PARAMETER

1. Pain or Burning Sensation (VISUAL ANALOG SCALE):



2. Mouth Opening: (Interinicisal Distance)

BEFORE TREATMENT	AFTER TREATMENT
……mm	……mm

HOMOCYSTEINE EVALUATION

BEFORE TREATMENT	AFTER TREATMENT
……µmol/L	…… µmol/L

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this day ----------between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai – 600 003, (hereafter referred to as, 'the college')

And

Prof Dr. G.V. MURALI GOPIKA MANOHARAN MDS., aged 50 years working as **Professor** in Department of Oral Medicine and Radiology at the Tamil Nadu Government Dental College, having residence address at Old No:3,New No:5,Avvaiyar Street, Nilamangai Nagar, Adambakkam,Chennai -600088 (Herein after referred to as 'Principal Investigator')

And **Dr. M.GAYATHRI** aged 29 years currently studying as **Post Graduate student** in Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College, residing at Valara, Natukkal (PO), Chittur (TK), Palakkad (DT), Kerala. Pincode-678554. (herein after referred to as the 'PG and co- Investigator').

Whereas the PG student as part of her curriculum undertakes this research on "**Evaluation of Serum Homocysteine as Prognostic Marker of Oral Submucous Fibrosis"** for which purpose the Guide shall act as Principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co- investigator.

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard.

Now this agreement witnessed as follows

1. The parties agree that all the Research material and ownership therein shall become the vested right of the college, including in particular all the copyright in the literature including the study, research and all other related papers.
2. To the extent that the college has the legal right to do go, shall grant to licence or assign the copyright so vested with it for medical and/or commercial usage of interested persons/ entities subject to a reasonable terms/ conditions including royalty as deemed by the college.
3. The royalty so received by the college shall be shared equally by all the three parties.
4. The Co-investigator and Principal Investigator shall under no circumstances deal with the copyright, Confidential information and know – how – generated during the course of research/study in any manner whatsoever, while shall sole west with the college.

5. The Co-investigator and Principal Investigator undertake not to divulge (or) cause to be divulged any of the Confidential information or, know – how to anyone in any manner whatsoever and for any purpose without the express written consent of the college.
6. All expenses pertaining to the research shall be decided upon by the Principal investigator/ Co-investigator or borne sole by the PG student (Co-investigator)
7. The college shall provide all infrastructure and access facilities within and in other institutes to the extent possible. This includes patient interactions, introductory letters, recommendation letters and such other acts requires in this regard.
8. The Principal Investigator shall suitably Guide Co-investigator the Student Right from selection of the Research Topic and Area till its completion. However the selection and conduct of research, topic and area of research by the student researcher under guidance from the Co-Investigator shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for the purpose.
9. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the Co-investigator, under the guidance from the Principal Investigator, the decision of the college may be binding and final.
10. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.

In witness whereof the parties hereinabove mentioned have on this day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its **Principal** **PG Student**

Witnesses **Student Guide**

1.

2.